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CONTRACTING ORGANIZATION: University of South Florida
                            Tampa, Florida   33620

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13. ABSTRACT (Maximum 200 Words)
The goals of the Advanced Cancer Detection Center include the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening and the testing of methods to prevent cancer. The projects included in this report are:

* Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (PI: Tockman)
* Development of the Moffitt Cancer Network (PI: Krischer)
* The Tampa Bay Ovarian Cancer Study (PI: Sutphen)

Each of these projects is presented as a complete study in the attached materials.

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INTRODUCTION:

The **Advanced Cancer Detection Center** (ACDC) of the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida received initial funding in October 1997. The creation of the Center followed a proposal that was developed in response to legislative language accompanying an appropriation in the Department of Defense budget that appeared in the September 28, 1996, Congressional Record:

"$3,500,000 is available only for the establishment of an advanced cancer detection center for military personnel, dependents, and retired service members, using a network that is in close geographic proximity and includes the following: a military hospital, a regional TRICARE provider, a Department of Veterans Affairs hospital or hospitals, and a medical facility with a focused cancer center that meets the National Cancer Institute eligibility requirements, with respect to research funding. The conferees would expect this center to conduct coordinated screening for cancer detection and treatment, to train military cancer specialists, and to develop improved cancer detection equipment and technology."

The ACDC at the H. Lee Moffitt Cancer Center and Research Institute has addressed these goals through studies that target the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening, and the testing of methods to prevent cancer. In addition, the ACDC created and supported education programs to provide increased cancer awareness, provide screening services, and has established working collaborations with the nearby James A. Haley VA Medical Center, the Bay Pines VA Medical Center and the MacDill Air Force Base Hospital.

The Advanced Cancer Detection Center supports research and demonstration projects that further its mission. Each project is reviewed for scientific merit by an internal peer group and an external scientific advisory committee. Preference is given to projects that have potential to lead to independent peer reviewed funding. During the current grant period, the ACDC supported eight cancer prevention and control research protocols. Four of these studies were continuations of projects begun in prior years and three are studies initiated the past year. The continuing studies are:

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (Cohort Study),

The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers,

The Tampa Bay Ovarian Cancer Study (TBOCS),

In November, 2000, both the internal and external scientific advisory committees met to consider the progress of the ACDC and to review and recommend new projects for funding. In this review, four additional projects were selected:

Epoxide hydrolase genetic polymorphisms and their functional significance, Automated Quantified Screening for Melanoma, Breast Cancer Screening in High-Risk Women: Comparison of magnetic resonance imaging (MRI) with mammography, and Adaptive Computer Assisted Diagnosis (CAD) Method for Lung Nodule Early Detection.

These studies were to be supported by FY00 and FY01 funding which was appropriated at the level of $3.5 million for each year. On September 20, 2001 the U.S. Army Medical Research and Material Command, (MRMC) Ft. Detrick, MD awarded the ACDC $6,004,000.00. According to the agency, this contract fully obligates the FY00 ($3.5M) and FY 01 ($3.5M) money directed to the ACDC by Congress. The remaining $996,000.00 (14.2%) is being withheld by the agency for "overhead expenses." The delay in the obligation of these funds was attributed to a complex "RCQ" (Regulatory Compliance and Quality) approval process by the agency over "human use" issues such as human genetics. Accordingly, progress on these projects has been delayed. With the exception of the <u>Epoxide hydrolase genetic polymorphisms and their functional significance</u> project (PI: Dr. J. Park), which was approved as human subject exempt, the remaining projects are still undergoing RCQ review. Progress reports on these studies will be reported under the new award made from the ACDC. Indeed, only the Cohort and TBOC studies will receive continued funding from this award in FY03. Final reports and publications are being readied on the remaining studies, as appropriate, or continuation funding is being developed under other mechanisms.

BODY:

<u>Overview:</u> The H. Lee Cancer Moffitt Center & Research Institute includes a free standing patient care facility with a large inpatient and outpatient capacity, a major research institute consisting of more than 130 scientific members, a free standing Lifetime Cancer Screening Center and a wide array of outreach and educational activities for the general public and select underserved populations. Moffitt Cancer Center's location at the convergence of the University of South Florida's Health Sciences Center and the main campus sets the stage for the its conceptual commitment to interdisciplinary approaches to research and patient care. Moreover, it allows the Center to enjoy all intellectual advantages of a matrix center while remaining operationally freestanding. After 14 years, the Cancer Center's mission remains totally focused on "contributing to the prevention and cure of cancer."

The Cancer Center was created by the Florida Legislature in the early 1980s, to meet a clear and compelling need to respond to Florida's "cancer epidemic." Building a major cancer research and treatment center at the University of South Florida in Tampa was largely the vision of H. Lee Moffitt, a state legislator who served as Speaker of the Florida House of Representatives from 1982-84.

Construction of the original, 380,000 square foot hospital facility was funded with $70 million from the state's cigarette tax, allowing the Center to open in 1986.

The initial phase of the Cancer Center's strategic plan called for a rapid and substantial deployment of its clinical, financial, and philanthropic resources to develop a true scientific center of excellence. The Center recruited Dr. John C. Ruckdeschel as the Cancer Center's first director in late 1991. In 1992, he began fulfilling that strategic plan, a process that culminated in the awarding of a Cancer Center Support Grant (CCSG) five years later.

The strategic plan's second phase continues the focus on scientific and clinical growth, with a commitment to increase research facilities by over 200,000 sq.ft., and to prepare to accommodate twice as many patients by 2009. In 1998, the state legislature committed an additional $100 million to finance the construction needed to meet these goals.

Key Milestones 1997-2001:

•Increased peer reviewed funding from $8 million in 1996 to over $20 million in 2001.

•Increased NCI funding from $4 million to $12.1 million.

•Recruited more than 20 new basic and physician scientists

•Hosted or co-hosted national and international cancer conferences, with a major conference, *New Molecular Targets for Cancer Therapy*, conducted on October 14-18, 2000.

•Opened 45,000 sq. ft. of additional laboratory space, with the help of an NIH construction grant (RR 13592) to complete one floor.

•Successfully competed for two program project grants: "Cancer Drug Discovery: Cell Cycle Control Targets," P.I.: Said Sebti, Ph.D. (1 P01 CA78038-01); and "Molecular Oncology Program Project," PI: William Dalton, Ph.D., M.D. (1P01 CA82533).

•Increased the number of patients enrolled on clinical trials (all types) from 1,809 in 1996 to more than 3,700 in 2000.

•Became the 17th member of the National Comprehensive Cancer Network.

•Worked with the University of South Florida to develop an Interdisciplinary Oncology Program (Department of Oncology) that will include most of the Cancer Center's faculty and that allows a distinct practice plan arrangement with the USF College of Medicine. This Department includes the entire basic science faculty

recruited by the Cancer Center and will be the academic home for a new interdisciplinary Ph.D. program in Molecular Oncology developed jointly by the Cancer Center and USF.

•Developed an Intellectual Property Sharing Agreement with the University of South Florida that gives the Cancer Center a percentage of all royalties and licensing fees on products developed by the Cancer Center's faculty.

•In September, 2000, the National Cancer Institute recommended renewal of the Cancer Center Support Grant for five years and the designation of Moffitt as an **NCI Comprehensive Cancer Center**, effective with its Notice of Award in February, 2001.

Today, the Cancer Center's membership numbers 130 scientists and clinicians who are USF faculty. More than 94 members-in-residence are housed and supported in the Center's facilities and work under the terms of the USF/Moffitt affiliation and faculty support agreements. Other members are based in University departments. The Cancer Center's 1,500 employees support the work of the physicians and scientists. The Center has annual operating revenues of over $130 million yearly, including an $11 million annual appropriation from the State of Florida, research grants totaling more than $26 million overall (direct), philanthropic donations, and institutional commitment from the University of South Florida in the form of faculty salaries and a portion of clinical practice revenues.

The Cancer Center currently supports four scientific programs:

Program	Leader	Members	Funding (Direct)
Molecular Oncology	Richard Jove, Ph.D.	21	$ 4,466,000
Immunology	Julie Djeu, Ph.D.	14	$ 2,486,000
Clinical Investigations	William Dalton, Ph.D., M.D.	58	$ 7,929,000
Cancer Control	Jeffrey Krischer, Ph.D.	39	$ 8,163,000
Non-aligned members & institutional grants	N/A	5	$ 3,089,000

The Cancer Control Research Program is the largest research program at Moffitt with 38 active members and more than $8 million in research funding. The overall goals of the Cancer Control Research Program remain focused on the reduction of the burden of cancer on individuals and society. The goals of the Cancer Control Research Program are translated into specific focused scientific aims that can be summarized as the application of multidisciplinary research to:

Aim 1 Susceptibility   Identify markers that predict increased cancer susceptibility.

Aim 2 Prevention	Evaluate promising interventions directed at the prevention of cancer.
Aim 3 Early Detection	Develop and testing new early detection strategies.
Aim 4 Health Outcomes	Evaluate interventions to improve the quality of life for cancer patients & their care-givers.

These aims are consistent with those of the Advanced Cancer Detection Center and the funding has been utilized to create an infrastructure to promote the goals of the Cancer Control Research Program by:

- •Encouraging collaborative research
- •Providing funding for studies that can lead to extramural peer-reviewed funding
- •Providing core competencies to support Cancer Control investigators

In order to provide an appropriate mechanism to allocate and manage these funds, the Cancer Center created an administrative core, an internal scientific review committee, and an external advisory committee. The administrative core manages the resources and personnel, associated with the ACDC funding, and provides liaison with the Department of the Army and the regulatory bodies that oversee the research. The internal scientific review committee conducts a scientific review of the merits of proposed projects and their potential for peer-reviewed funding and makes funding recommendations. The external advisory committee reviews the organizational structure and scientific directions of the Advanced Cancer Detection Center and the progress made by the individual projects.

The membership of the internal scientific review committee changes as necessary to have adequate scientific expertise to evaluate proposals submitted to the ACDC. This year the members are:

Dr. Dmitry Goldgof, Associate Professor, Computer Science and Engineering, College of Engineering
Dr. Pamela Munster, Assistant Professor, Department of Interdisciplinary Oncology, College of Medicine
Dr. Santo Nicosia, Professor and Chair, Department of Pathology, College of Medicine
Dr. Robert Clark, Professor and Chair, Department of Radiology, College of Medicine
Dr. Jeffrey Krischer, ex officio, Professor, Department of Interdisciplinary Oncology, College of Medicine

Cancer Control science at the H. Lee Moffitt Cancer Center and Research Institute is greatly enhanced and facilitated by the development of infrastructure that provides access to shared resources, promotes collaboration and funds pilot projects. Over the last three years, the Cancer Control Program has established new infrastructure to meet these needs. The funding of the Advanced Cancer Detection Center is one of three mechanisms by which this has occurred.

Advanced Cancer Detection Center

The Advanced Cancer Detection Center has become a significant component of the Moffitt Cancer Control Program infrastructure in that provides a stimulus for research development and promotes inter and intra programmatic collaborations. The Advanced Cancer Detection Center supports pilot studies that can lead to peer-reviewed extramural funding. Projects supported by this mechanism follow a two-tiered scientific review process in which the science and the likelihood of peer-reviewed extramural funding are considered. In addition, priority is given to projects that foster inter and intra-programmatic collaborations.

Center for Mathematical-Modeling of Image Data Across the Sciences (MIDAS) (PI:Lazaridis), through a two-year competitive grant jointly funded by

the Moffitt Cancer Center and the University provost's office in June, 1999. The mission of the MIDAS center is to create interdisciplinary collaborations among imaging, quantitative and biological scientists, in order to develop new analytic models of image-related data and to train researchers of various disciplines in modeling techniques.

Figure 1: MIDAS Projects
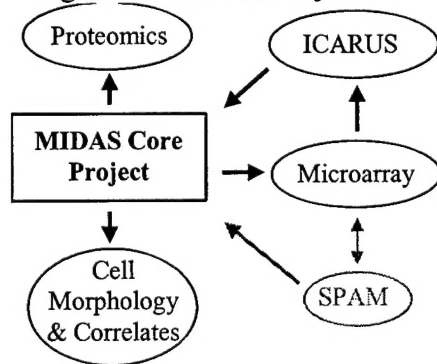
The Center is comprised of a core project and several satellite projects. The core project is to construct a data analytic environment that builds advanced statistical methodologies on top of available imaging componentry. By borrowing and uniting technologies from multiple fields, we are seeking to empower researchers in both basic and clinical imaging studies with a sophisticated analytic toolbox. Because this core project is a prerequisite technology for a center focused on quantitative, collaborative analyses of image-related data, substantial resources have been committed to its completion, including hardware, software, and the efforts of two statistical assistants and data systems programmers.

Multiple Java interfaces and servlets are being built from reusable object-oriented code to tie together powerful statistical and database systems (Figure 2). Graduate students and postdoctoral fellows participating in the satellite projects also contribute to the core project. New methods have been developed and a patent application is pending. The applied projects entail interactions with investigators in Molecular Oncology, Clinical Investigations as well as Cancer Control.

Figure 2: MIDAS Core Project Design
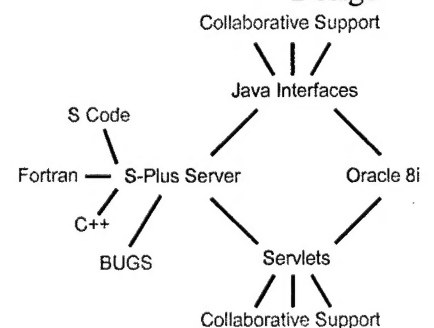
Moffitt CCOP Research Base (PI:Krischer)

The H. Lee Moffitt Cancer Center received funding by the NCI in June 2000 to develop a research base as a mechanism for Community Clinical Oncology Programs to access cancer control clinical trials. NCI CCOPs and Moffitt affiliates are eligible to participate in the Moffitt CCOP Research Base. Membership is based on continued funding as an NCI CCOP with satisfactory performance measured by accrual and data quality.

The goals of the Moffitt CCOP Research Base are to:
- Develop cancer control trials of high scientific merit for implementation in the community setting.
- Provide community investigators an opportunity to participate in NCI-supported cancer control clinical trials.

The following CCOPs have, or are in the process of, establishing formal affiliations with the Moffitt CCOP research base:
Florida Pediatric CCOP, Tampa, FL
Merit Care Hospital CCOP, Fargo, ND
Mount Sinai Medical Center CCOP, Miami, FL
South Texas Pediatric MBCCOP, San Antonio, TX
Baptist Center Research Institute CCOP, Memphis, TN
Cancer Research for the Ozarks CCOP, Springfield, MO
Columbus CCOP, Columbus, OH
Greater Phoenix CCOP, Phoenix, AZ
North Shore University Hospital CCOP, Manhassett, NY
NorthWest CCOP, Boise, ID
Southern Nevada Cancer Research Foundation CCOP, Las Vegas, NV

The Moffitt CCOP Research Base is now staffed and cancer control protocols and concepts are being initiated. Several of the clinical studies are the result of pilot development funded by ACDC projects:

A Clinical Trial of the Action of Isoflavones in Breast   Protocol   Pending 10/17/2001
Neoplasia: Administration Prior to Mastectomy or
Lumpectomy -- A Pilot Study

The Specific Role of Isoflavones in Reducing Prostate   Protocol
  Activated   09/05/2001
Cancer Risk

A Randomized Pilot Clinical Trial of the Action of   Protocol   Pending 09/06/2001
Isoflavones and Lycopene in Localized Prostate Cancer:
Administration Prior to Radical Prostatectomy.

Megestrol Acetate (Megace) as an Appetite Stimulant in Activated Children 02/27/2001	Protocol	
Cancer Genetic Counseling and Testing by Telemedicine in Community Settings	Concept	Pending
Methylphenidate for the Treatment of Cognitive Difficulties in Patients with Primary Brain Tumors: A Double-Blinded, Placebo-Controlled, Parallel Group Study	Concept	Pending
Stress Management Training for Patients Undergoing Radiotherapy	Protocol	Pending

(1) KEY RESEARCH ACCOMPLISHMENTS:

The material that follows in this section summarizes the key research accomplishments associated with each project and task outlined in the appropriate approved Statement of Work for ACDC approved projects. **A full description of the projects and their progress is appended.**

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers, Genetic Analysis of Familial Prostate Cancer

- Developed an infrastructure to identify, accrue, screen and follow a non-diseased community-dwelling population at high risk for lung cancer.

- Developed procedures for collection and preservation of sputum specimens for new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of airways cytologic specimens suitable for evaluation of new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of white blood cells suitable to provide individual control specimens for DNA and RNA

- Developed a potency assay for MoAb 703D4 immunodetection of hnRNP A2/B1 protein expression.

- Identified a panel of 15 LOH markers for sputum lung cancer screening which identifies 84% of lung tumors.

- Completed the initial lung cancer screeing of 1151 middle-aged current and former smokers. Approximately 50% are found in stage I, a 3-fold greater

frequency of stage I detection compared to the current clinical standard of no screening as reported by the Florida Cancer Registry.

The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers

- The Moffitt Cancer Network is available to users and can be found at
  http://network.moffitt.usf.edu
- The MCN currently has 576 presentations in its library, increasing at a rate of 16 presentations per month on average. Additionally, 16 conferences sponsored by USF and Moffitt are also currently available online.
- All approved Grand Rounds presentations have been taped by the Moffitt Multimedia Education Resources Center (MERC) for over two year preceding this report. The video was previously captured on digital DVCAM 94 minute tapes. Currently we are running in a tape-less environment.
- Since many of the presenters use only 35mm slide for their presentations, a process of creating final production audio/video Real media for streaming via TCP/IP has been developed. This process requires post-production labor and requires the best of the video's individual frames to be captured a second time to recreate higher quality computer images. MCN has made significant progress in this area and as of June 2000 has begun using presenter's PowerPoint files when ever possible to bypass the second image rendering process. This has reduced labor time from 3.5 days to about 5 hours, while increasing image quality noticeably. This labor savings is not realized when presenters are using 35mm film only. This methodology was modified to capture slides, overheads and computer screens digitally without a camera. The new methodology has reduced post-production time to virtually nothing. This allows us to concentrate on acquisition of new material.
- In addition to pre-presentation file acquisition, MCN has begun the development of a presenter packet. When finished, this packet will inform presenters to repeat important questions asked at the end of events like Grand Rounds and these will be added to the content to be available to medical professionals at the MCN website.
- National oncology conferences have been taped and included in the MCN website database.
- Conferences have been subdivided into their respective presentations and are categorized searchable as well as searchable using the website database Access Jet engine. All conferences are pre-qualified for their ability to become online educational materials by the University of South Florida College of Medicine and, more recently, the University of South Florida College of Nursing.
- MCN began simultaneous live streaming and archiving in late 2001. This process greatly reduces postproduction time while increasing access to live events.
- MCN has completed the move to camera-less and tape-less acquisition of presentations using a host of digital equipment.

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The Tampa Bay Ovarian Cancer Study

- Aim 1: Data regarding health behaviors and risk factors were obtained from all participants via questionnaire instruments and study interview was successfully completed on all 231 women. Data regarding menopausal status has been compiled on 138 women. Of the 13 mutation carriers on whom data has been compiled, 0, 4, and 9 are pre-, peri-, and post-menopausal, respectively. Of the 125 sporadic cases on whom data has been compiled, 17, 26, and 82 are pre-, peri-, and post-menopausal, respectively.
- Aim 2: Detailed cancer family history was obtained from all participants via questionnaire instruments and study interview.
- Aim 3: A successful mechanism has been implemented to obtain medical records and tumor tissue in order to compare tumor characteristics between mutation-associated cases and non-mutation controls.
- Aim 4: A successful follow-up mechanism has been implemented to obtain data regarding differences in response to treatment and survival between mutation-associated cases and non-mutation controls.
- Aim 5: Although we were not able to achieve an 80% participation rate, we have been able to accrue a population-based sample with regard to ethnicity, stage, histologic subtype, median age and ethnicity (refer to Tables 2 and 3). Based on Florida Cancer Data System (FCDS) data, we estimate that a total of 430 patients were diagnosed with ovarian cancer during the study period, of whom 350 patients were ascertained (ie: 82%). There were 12 patients who died prior to enrollment, 18 patients or doctors declined, and a further 10 patients who could not be enrolled prior to the closing of the study. Hence of the 350 cases ascertained, 231 were enrolled in the study (66%).

(2) REPORTABLE OUTCOMES:

Manuscripts, abstracts, presentations:

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers

Publications Related to this Study:

1. Bepler G, Goodridge Carney D, Djulbegovic B, Clark RA, **Tockman M**. A systematic review and lessons learned from early lung cancer detection trials using low-dose computed tomography of the chest. Cancer Control. 2003; 10:306-14.

2. Toyooka S, Toyooka KO, Miyajima K, Reddy JL, Toyota M, Sathyanarayana UG, Padar A, **Tockman MS**, Lam S, Shivapurkar N, Gazdar AF. Epigenetic down-regulation of death-associated protein kinase in lung cancers. Clin Cancer Res. 2003; 9:3034-41

3. Zhukov TA, Johanson RA, Cantor AB, Clark RA, **Tockman MS**. Discovery of distinct protein profiles specific for lung tumors and pre-malignant lung lesions by SELDI mass spectrometry. Lung Cancer. 2003 ;40:267-79.

4. Ratnasinghe DL, Yao SX, Forman M, Qiao YL, Andersen MR, Giffen CA, Erozan Y, **Tockman MS**, Taylor PR. Gene-environment interactions between the codon 194 polymorphism of XRCC1 and antioxidants influence lung cancer risk.
Anticancer Res. 2003; 23:627-32

5. Baker SG and **Tockman MS**. *Evaluating Serial Observations of Precancerous Lesions For Further Study as a Trigger For Early Intervention.* Statistics in Medicine, 2002; 21:2383-90.

6. Toyooka S, Fukuyama Y, Wistuba II, **Tockman MS**, Minna JD, Gazdar AF. *Differential Expression of FEZ1/LZTS1 Gene in Lung Cancers and Their Cell Cultures.* Clinical Cancer Research. 2002 July; Vol 8 2292-2297.

7. Chirikos T, Hazelton T, **Tockman MS**, and Clark R. *Screening for Lung Cancer with CT: A Preliminary Cost-Effective Analysis.* CHEST 2002; May 121:1507-14.

8. Zhou J, Nong L, Wloch M, Cantor A, Mulshine JL, **Tockman MS**. *Expression of early lung cancer detection marker: hnRNP A2/B1 and its relation to microsatellite alteration in non-small cell lung cancer.* Lung Cancer. 2001; Dec 32(3):341-50.

9. Mulshine JL, De Luca LM, Dedrick RL, **Tockman MS**, Webster R, Placke ME. *Considerations in developing successful, population-based molecular screening lung cancer.* Cancer. Dec 1, 2000; 89(11 Suppl):2465-7. Review.

10. **Tockman MS**, Mulshine JL. *The early detection of occult lung cancer.* Chest Surg Clin N Am. Nov 2000; 10(4):737-49.

11. **Tockman MS**. *Advances in Sputum Analysis for Screening and Early Detection of Lung Cancer.* Cancer Control, Journal of the Moffitt Cancer Center. January/February 2000, Vol. 7, No. 1:19-24.

12. Kennedy TC, Proudfoot SP, Piantadosi S, Wu L, Saccomanno G, Petty TL, **Tockman MS**. *Efficacy of Two Sputum Collection Techniques in Patients with Air Flow Obstruction.* ACTA Cytologica. July-August 1999; Vol. 43, No. 4:630-636.

Abstracts Related to this Study

1. Zhukov TA, Johanson R, **Tockman MS**. *Discovery of distinct protein profiling specific for lung tumors and pre-malignant lung lesions by SELDI*

mass spectrometry. Proceedings of the AACR, Volume 43, March 2002. Page 36, #184.

2. Zhukov TA, Erozan YS, **Tockman MS.** *Sentinel Cell for Lung Cancer.* 14th International Congress of Cytology, RAI Congress Centre, Amsterdam, The Netherlands. May 2001.

3. Mulshine JL, **Tockman MS**, Martinez A, Man Y-G, Montuennga L, Hong SH. *Application of Molecular Biology for Early Detection of Lung Cancer.* 1999.

4. Zhou J, Nong L, Wloch M, Zhukov TA, and **Tockman MS.** *Expression of Early Lung Cancer Detection Marker: hnRNP a2/b1 and its Relation to Microsatellite Instability in Non-Small Cell Lung Cancer.* Proc. AACR, April 1999, 40:140-141

5. Truncale T, Zhou J, Zhukov TA, Muñoz-Antonia T, Antonia S, Muro-Cacho C, **Tockman MS.** *Evaluation of TGF-ß II Receptor Expression (TßR-II) and a Common Signaling Mediator SMAD4 in NSCLC.* Proc. AACR, April 1999, 40:337.

6. Falestiny MN, Cardona JJ, Zhou J, Zhukov TA, Nong L, Solomon DA, **Tockman MS.** *Transforming Growth Factor ß Type II Receptor Expression in Non-Small Cell Lung Cancer, Viral Transformed Bronchial Cells and Normal Bronchial Epithelial Cells: A Comparative Study.* Chest (Suppl); November 1998.

7. **Tockman MS**, Saccomanno G, Michels R, Zhukov TA, Erozan Y, and Gupta P. *Sentinel Cell for Lung Cancer.* 6th SPORE Investigator's Workshop; July 1998.

Presentations Related to this Study:

December 8-10, 1998   ***International Conference on Prevention and Early Diagnosis of Lung Cancer**, Johns Hopkins Lung Project* and *Immunocytochemical Screening for Lung Cancer.* University of Varese and University of Massachusetts Medical School, Varese, Italy.

February 12, 1999   ***ALCASE Workshop – Lung Cancer: A Revolution in Care**, Technology in Early Diagnosis of Lung Cancer.* Embassy Suites, Tampa, Florida

April 26, 1999   ***1999 ALA/ATS International Conference Program**, Early Sputum Marker for Lung Cancer (hnRNP).* San Diego Convention Center, San Diego, California

April 30, 1999   ***Pharmacology Seminar Program***, *Detection and Immunostaining of the Lung Cancer Sentinel Cell.* University of Pittsburgh, Pennsylvania.

September 13, 1999   ***Advanced Cancer Detection Center***, *External Advisory Committee.* Moffitt Cancer Center, Tampa, Florida

September 30th to October 3, 1999   ***The First International Conference On Screening for Lung Cancer,*** Cornell University, New York

October 9-13, 1999   ***Annual Congress of the European Respiratory Society,*** *Dysregulation of the Cell Cycle in Lung Cancer.* Madrid, Spain

October 15-16, 1999   ***Molecular Biomarkers Workshop***, Roy Castle Lung Cancer Foundation, Liverpool, England

October 26, 1999   ***Screening of Lung Cancer Conference***, Gaithersburg, MD

October 31, 1999   7th Annual Scientific Assembly of the American Association of Bronchology, *New Horizons in Cytological Based Early Detection in Lung Cancer.* Chicago, IL

February 9, 2000   ***International Symposium on Early Detection of Lung*** Cancer, *Molecular Screening Program: Past, Present, and Future.* Tel Aviv, Israel

February 27-29, 2000   ***International Agency for Research on Cancer, Use of Biomarkers in Chemoprevention of Cancer,*** *Lung Cancer: Intermediate Effect Markers.* Heidelberg, Germany

March 20, 2000   ***Cahan Lectureship at Memorial-Sloan Kettering***, *Molecular Screening for Lung Cancer.* New York, NY

April 12, 2000   ***Early Detection Research Network Site Visit at H. Lee Moffitt Cancer Center & Research Institute,*** *Organization of BeDLAM.* Tampa, FL

June 16, 2000   ***Wayne State University Cancer Conference,*** *Sputum in 2000: Hypothetical Advantages, Practical Limitations, and Novel Approaches,* Detroit, MI

June 22, 2000   ***Reducing Lung Cancer Mortality: Actions for the New Millenium,*** *Sputum Based Detection of Preinvasive Lung Cancer,* Washington, DC

June 27, 2000   **Roy Castle Lung Cancer Foundation and H. Lee Moffitt Cancer Center, Quest for the Cure,** *Lung Cancer Screening and Early Detection: Spiral CT Scanning and Molecular Markers,* Tampa, FL

July 19, 2000   **H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,** *Epidemiology and Early Detection of Lung Cancer,* Coeur d'Alene, ID

July 19, 2000   **H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,** *The Management of Pre-Clinical Lung Cancer,* Coeur d'Alene, ID

September 12, 2000   **IASLC 9th World Conference on Lung Cancer,** *Cellular Targeting in the Molecular Diagnosis of Lung Cancer,* Tokyo, Japan

October 24, 2000   *66th International Scientific Assembly of the ACCP,* San Francisco, CA

                     ACCP Post Graduate Course, **Screening and Early Detection of Lung Cancer**

                     Meet the Professor, **Sputum Detection of Early Lung Cancer: Hypothetical Advantages, Practical Limitations, and Novel Approaches**

October 27, 2000   **Cornell CT Conference,** *Sputum Detection of Early Lung Cancer: A Compliment to Helical CT,* New York, NY

March 7-8, 2001   **Lung Cancer Early Detection Workshop,** National Cancer Institute/American Cancer Society, **"New Frontiers of Screening Science".** Rockville, MD

March 24-25, 2001   **Second Annual – A Practical Pulmonary Review for Primary Care Providers,** University of South Florida/Department of Veterans Affairs, James Haley, **"Early Recognition of Lung Cancer".** St. Pete Beach, FL

June 20-22, 2001   **Early Detection Research Network,** National Institute of Health/ National Cancer Institute, *"Lung Cancer Screening Update"* and *"Industrial Partnership with EDRN",* Washington, DC

June26-July2, 2001	***Second International Lung Cancer Molecular Biomarkers Workshop "A European Strategy for Developing Lung Cancer Molecular and Clinical Diagnostics in High Risk Populations,*** Roy Castle Lung Cancer Foundation, *"Sputum in 2001: Hypothetical Advantage Practical Limitations, and Novel Approaches"* and *"Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers",* Liverpool, England
August 7-12, 2001	***3rd International Conference on Prevention & Early Detection of Lung Cancer,*** International Association for the Study of Lung Cancer, *"Cellular Approaches to Lung Cancer Detection"* and *"Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers".* Rejkjayik, Iceland
October 13-17, 2001	EDRN Scientific Workshop, Seattle, WA
October 26-29, 2001	5th International Conference on Screening for Lung Cancer, New York, NY
December 5-6, 2001	NCI Grant Review Meeting RFA CA-02008 Chemoprevention of Tobacco-related Cancers in Former Smokers: Preclinical Studies, Washington, DC
February 3-5, 2002	EDRN Steering Committee, Houston, TX
March 10-15, 2002	New Frontiers in Cancer Detection & Diagnosis (EDRN/ Gordon Research Conference), Ventura, CA
April 5-7, 2002	6th International Conference on Screening for Lung Cancer, Paris, France
June 13, 2002	NIH Women Tobacco & Cancer Steering Committee, Bethesda, MD
June 22, 2002	Great Cancer Roundup: Lung Cancer Screening & Prevention Conference, Los Angeles, CA
September 3-5, 2002	6th EDRN Steering Committee, Ann Arbor, MI
October 11-15, 2002	Molecular Targets in Cancer Therapy, St. Petersburg, FL
October 18-20, 2002	7th International Conference on Screening for Lung Cancer, New York, NY
October 28-30, 2002	1st International Lung Cancer Conference, Beijing, China

The Tampa Bay Ovarian Cancer Study

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was awarded by the American Cancer Society for a companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer (7/1/00 – 6/30/04). Preliminary data is promising and shows that certain lysophospholipids appear to be elevated in the plasma of women with ovarian cancer compared with healthy controls (article accepted in Cancer Epidemiology, Biomarkers, and Prevention and included in Appendix A). We have applied to ACS for a two-year extension of the project. Also, based on this preliminary data, we have applied to NIH for R01 funding to investigate the use of lysophospholipid measurement and proteomic profiles for detection of ovarian cancer in a case-control study.

Based on data showing that gene mutations associated with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are the third leading cause of hereditary ovarian cancer (after BRCA1 and BRCA2), and the suggestion that ovarian cancer is a "sentinel cancer" in individuals with these gene mutations, an investigation of HNPCC as a companion study of TBOCS has been funded.

The preliminary results of this research were presented at the 2002 American Society of Human Genetics annual meeting (Appendix B), and 2003 Frontiers in Cancer Prevention Research (American Association for Cancer Research) annual meeting (Appendix C).

The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Provider

Patents and licenses applied for and/or issued:

> A notice of disclosure has been filed with the USF office of patents in anticipation of the completion of a patent application.

Presentations:
- The Moffitt Cancer Network Vision, Jeffery Krischer, Ph.d. April 2001
- The Moffitt Cancer Network, Lessons Learned and New Directions, Matthew Clark, B.S. October 2001
- The Moffitt Cancer Network 2002, Matthew Clark, B.S. April 2002
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., May 19, 2002 Medical Library Association Conference, Dallas TX
- No-latency video architecture, efficiency and a new tomorrow for on-line education, Matthew Clark, B.S. June 2002
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S.,June 19, 2002 Tech Topics, Moffitt

- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S.,October 19, 2002 Southern Chapter, Medical Library Association
- Disseminating Library Instruction to the Desktop via the Web, Sue Felber, M.S.,October 19, 2002 Southern Chapter, Medical Library Association
- Telemedicine Today and Tomorrow, Matthew Clark, B.S. October 2002

Abstracts:
- J Permuth-Wey, JA Betts, AB Cantor, JP Krischer, R Sutphen: Cancer Genetic Counseling and Testing by Telemedicine - Results of a Feasibility Study (Abstract). American Journal of Human Genetics (2002) 71(4): 343.

- **Funding received based on work supported by this award:**

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers

1. "The Biomarker Development Laboratory at Moffitt" (NCI-CA 84973, M. Tockman, PI, 1st year/Total award $413,720/$1,903,827).
2. "Identification of the Lung Cancer Epitope Identified by the Monoclonal Antibody 703D4" (Cancer Research Foundation Of America, M. Gruidl, PI, Total Award $38,950)
3. J. Park, PI, NCI-EDRN, Total award $98,000.

The Tampa Bay Ovarian Cancer Study

- Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was awarded by the American Cancer Society for a 3-year companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer. Preliminary data is promising and shows that certain lysolipids appear to be elevated in the plasma of women with ovarian cancer compared with healthy controls. Based on this preliminary data, we have applied for an R01 to investigate the use of lysolipid measurement for detection of ovarian cancer in a population of women at increased risk of ovarian cancer, including first-degree relatives of women in TBOCS (ovarian cancer patients). Our ongoing contact with women in TBOCS will facilitate the identification and enrollment of their female relatives at increased risk for enrollment in this important study, toward the development of an early detection test for ovarian cancer.

- Based on the promising preliminary results from our current investigations of lysolipids as biomarkers of ovarian cancer, we are also seeking funding from NCI for an investigation of these substances for their use as markers of recurrence of ovarian cancer, in a community-based investigation of ovarian cancer patients.

(3) CONCLUSIONS:

The Advanced Cancer Detection Center has been a great success. It has attracted quality research projects from among Cancer Center members, it has promoted inter and intra programmatic research and its projects have begun to lead to peer-reviewed extramural funding. Two of these studies (Selenium, DAMD 17-00-1-0062, and the Moffitt Cancer Network, DAMD 17-00-1-0055) received independent peer-reviewed funding from the Department of Defense in February, 2000. Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study supported by this award, funding was awarded by the American Cancer Society for a 3-year companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer. The study of markers of transformation in airways epithelial cells from a cohort of obstructed smokers and former smokers supported the establishment of the Biomarker Development Laboratory at Moffitt also funded by the National Cancer Institute, (PI: M. Tockman,). The Cohort Study is the only study in the nation that currently evaluates both molecular airways markers and helical CT examinations simultaneously in the prospective detection of lung cancer. Until the Cornell and Mayo studies begin their collection of sputum specimens, no other study addresses the relative merits of these apparently complementary techniques for lung cancer screening. This research question addresses the most common cause of cancer death and the only common cancer for which no screening is available. Finally, the archive of radiographs, sputum and blood cell specimens provides an infrastructure for other investigators at Moffitt and across the nation. The recent award to Dr. Jong Park of an NCI Early Detection Research Network grant was based on the availability of the Cohort archive.

The current funding period has been extended to permit the successful conclusion of projects that are still in the accrual or analysis phases. Manuscripts, presentations and grant proposals are under development to communicate the results of these efforts widely and to secure additional funding to pursue promising findings. The Moffitt Cancer Network is a functioning, stable educational forum and entering its evaluation phase. New funding has been put in place to continue the efforts of the Advanced Cancer Detection Center and to transform it into a DoD Center of Excellence to recognize its capability to identify and fund outstanding research from the entire Cancer Center.

(4) REFERENCES:

References pertinent to the individual projects are contained in the appended material.

Appendix A

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers

Melvyn Tockman, M.D., Ph.D.

Markers of Transformation in Airways Epithelial Cells
from a Cohort of Obstructed Smokers and Former Smokers
(DoD Cohort Study)
Final Report

September 30, 2003

I. Introduction
Lung Cancer Screening with Helical CT and hnRNP A2/B1

Computerized molecular analysis of airway cell markers (ACM) and helical computed tomography (CT) may be evaluated in targeted populations for detection of the earliest signs of lung cancer. The objective of this study is to determine whether application of these screening techniques in a high-risk population will result in a 3-fold improvement in stage at diagnosis (stage-shift) from the current 20% in stage 1 (Florida Cancer Registry) to 60% in stage 1. At the conclusion of this study, we will compare the accuracy (sensitivity and specificity) as well as the stage distribution of lung cancer detected by these methods. We have completed the accrual required for this study, submitted our report of the Moffitt prevalence experience with helical CT screening for publication (attached as Appendix) and are preparing our report of the first annual (incidence) screening.

Henschke et al (Lancet, 1999 354:99-105) found that 10% of helical CT-detected noncalcified nodules from 2-5 mm through 21-45 mm contained a primary lung cancer. This is four times the sensitivity of a standard chest x-ray taken at the same time. Our preliminary data (Clin Cancer Res, 1997, 3:2237-46) showed that computerized immuno-detection of up-regulated hnRNP A2/B1 expression in sputum cells detected primary lung cancer in 37 of 45 (82%) cases. This is 8 times the sensitivity of standard sputum cytology obtained at the same time.

It is quite likely that the helical CT and ACM protein expression screening are complementary. The cell type distribution of the detected cancers suggests this. Lung cancer cell types infrequently detected by helical CT (squamous and small cell) may be detected by ACM. Evaluation of the extent to which these early lung cancer detection techniques are complementary could only be conducted in a prospective cohort study such as this one, where every individual is screened by both techniques at the same examination.

Other Airway Cell Markers of Lung Cancer Evaluated with Cohort Specimens

In parallel, our NCI-EDRN Biomarker Developmental Laboratory at Moffitt (BeDLAM) grant supports the examination of other potential molecular markers on specimens archived from this DoD-Cohort trial. Several years ago with David Sidransky at Hopkins, we pioneered the use of microsatellite alterations as clonal markers in the detection of human cancer (Proc Natl Acad Sci USA 1994; 91:9871-75). We have found that microsatellite alteration and LOH on 3p is significantly associated with upregulation of hnRNP A2/B1 (Proc. AACR 1999; 40:140-1 Lung Cancer. 2001; Dec 32(3):341-50). Further, loss at 3p22, the site of gene for the Type II Transforming

Growth Factor Beta Receptor is strongly associated with NSCLC (Clin Cancer Res. 2001 Jun;7(6):1618-26). Altered messenger RNA and proteins of the downstream tumor suppressor TGF-ß signaling pathway are of great interest in our laboratory (Clin Cancer Res. 2001 Jun;7(6):1618-26). BeDLAM funding also supports evaluation of the extent to which gene-specific promoter hypermethylation, detected in archived sputum cells, predicts the development of lung cancer. We have received supplemental NCI-EDRN funding to develop a lung cancer methylation array on which Cohort sputum specimens may be evaluated. Therefore, we have developed a technique for preservation of sputum morphology and nucleic acids so that (DNA) promoter hypermethylation and microsatellite alterations as well as altered TGF-ß type II receptor message expression (RNA markers) may be examined in the sputum specimens collected in this study.

Ventilatory Obstruction (impaired spirometry) Enhances the Lung Cancer Risk of Cohort Participants

Age and cigarette smoking are not the only lung cancer risk factors considered in this study. We have shown that current and former smokers distinguished by airways obstruction are at 2-4 fold risk of developing lung cancer compared to non-obstructed smokers (Ann Int Med 1987:106:512-8). This result has been corroborated in the Multiple Risk Factor Intervention Trial, a randomized clinical trial for the primary prevention of coronary heart disease that enrolled 12,866 men. In that study, ventilatory function was a powerful predictor of lung cancer deaths, with rates that increased from 3.02 per 1,000 person-years in the lowest quintile of forced expiratory volume to 0.43 in the highest quintile (Am J Epidemiol. 1990 Aug;132(2):265-74). A similarly high lung cancer frequency among obstructed current and former smokers has been observed more recently among the participants in the Colorado Lung Cancer SPORE (Cancer Res. 1996 Oct 15;56(20):4673-8).

Former Smokers Remain at Risk of Lung Cancer and Could Benefit from Screening

The populations of greatest interest for lung cancer screening are the estimated 46 million former smokers in the United States who remain at risk despite smoking cessation. While cardiovascular risk resolves rapidly, Wistuba et al. have shown that genetic alteration of airway lining cells observed in current smokers is not reversed in former smokers (JNCI 1997; 89:1336-73). Progression to lung cancer is probably only slowed, but not reversed by removal of the promotional stimuli of smoking. Major medical centers (Beth Israel, MD Anderson, Cancer 1996; 78:1004-10) now report similar numbers of new lung cancer cases from former as from current smokers. Former smokers, having followed the cessation advice of the medical establishment, remain at risk of lung cancer and are likely to benefit greatly from validated lung cancer screening (Cancer. 2000 Dec 1;89(11 Suppl):2506-9).

Body

Start-up: Study Activation, 0-3 Months

Approval: The protocol, informed consent and data collection forms were completed and this study was approved by Moffitt/USF IRB on November 5, 1998, with

conditional approval by Army Regulatory Compliance on December 23, 1998. The protocol was resubmitted with amendments covering novel methods of sputum preservation to the Moffitt/USF IRB and received Army Regulatory Compliance final approval and study activation on June 10, 1999.

Space Renovation: Two spirometry/sputum induction facilities were originally established. The facility at the Lifetime Cancer Screening (LCS) Center is fully operational. This facility includes a spirometry screening station, a laminar-flow sputum induction hood, and a biosafety cabinet for sputum specimen processing. Interviews and blood drawing also take place in this space. During 2001, a helical CT scanner was installed so that the entire Cohort screening process now can take place at LCS. The screening station at the James A Haley VA Hospital was used primarily for spirometry screening of volunteers identified through the VA Respiratory Division. A large number (n=1259) of Respiratory Division patients were screened and we have now exhausted that patient population for potential study participants. During 2000, the screening station at the James A Haley VA Hospital was closed.

Equipment Purchased: At start-up, several major pieces of equipment were purchased to support this study. These include a Helical CT scanner, a Perkin-Elmer 310 gene scanner, and an Arcturus PixCell II Laser Capture Microdissector. Several minor pieces of equipment have also been purchased, including an induction safety cabinet, nebulizer, and a sputum preparation biosafety hood. In the past two (2) years, no new equipment has been purchased for this study.

<u>Recruitment Phase, 3-24 Months</u>
Staff Hired: At present, only 1.34 FTE's are funded by DoD to partly support the administrative secretary and the study nurse for participant scheduling and clinical follow-up. The study coordinator and clinical research associate are funded by BeDLAM to oversee the annual follow-up (incidence) screening, data management, and specimen collection.

Accrual: The study has met its accrual goal of 1150 eligible subjects $\geq$ 45 years of age with $\geq$ 30 pack years of smoking who have been screened by spirometry (Tables 1 & 2). Our prior sputum/CXR screening trials have shown that in males of this age range with this smoking history, clinical lung cancer will have a 0.7% (7/1000) prevalence and 0.5% (5/1000) annual incidence. In the presence of mild obstruction, the annual lung cancer incidence increases to 1.1% (11/1000) and continues to rise with increasing obstruction. After four (4) screening examinations and estimating a 23% prevalence of obstruction in the study population, we would have predicted 44-50 cases of lung cancer (11-13 cases per year).

At the conclusion of accrual, 3,496 individuals had been screened, 1151 of whom have been enrolled and undergone sputum induction and helical CT screening. Thirty-eight percent of the screened population has been referred for evaluation from the Respiratory Clinic at the James A. Haley VA hospital. This pool of recruits has more ventilatory obstruction than the general population. Exceeding the 23% rate of mild

obstruction expected from a (non-clinic) population of cigarette smokers, we find that 59% of our screened population meet the obstruction criterion. By designing the study to include younger, obstructed participants, we have indeed accrued a population at high risk for lung cancer. The observed lung cancer prevalence of 2.3% is more than double that expected among the 1151 first examinations, and the incidence of 0.45% is approximately the same as expected among the 889 follow-up examinations. If this trend continues, 57 cases would be expected to develop in this population by the end of the study, 14% greater than the required sample size.

Prevalence Results, Demography: The study population currently consists of 1151 participants who are on-study and were included in the tabulation below. From this population 682 (59%) were obstructed. Twenty-seven (27) of 1151 participants have developed lung cancer (prevalence 2.3%). Twenty-three (85%) of 27 prevalent cases were obstructed. Those who developed cancer were white, 44% were male, with an average 59.7 pack years of smoking and an FEV_1 /FVC of 62%. The age, race, and gender distributions of the cancer cases do not differ from that of the obstructed or total screened populations (Table 3). As might be expected, the medical histories of the obstructed population (and cancer cases) more frequently report the presence of chronic lung diseases. Preliminary comparison of occupational/environ-mental exposures shows no important differences (Data not shown).

Incidence Results, Demography: To date, a total of 1455 follow-up screening visits have been completed (Table 4). During the follow-up visits, eleven cancer cases have been detected during the first, second, and third follow-up visits. All eleven cancer cases were obstructed (mean FEV_1/FVC of 52.4%) with a mean pack smoking history of 79.5 pack years. Ninety-one percent of the incident cancer cases were white.

Preliminary Results, Radiographic Screening: Four hundred and six (35.2%) of 1151 initial and 174 (26.9%) of 646 follow-up helical CT scans have shown an abnormality (non-calcified nodule). Thirteen (48%) of 27 prevalence and 6 (67%) of 9 incidence lung cancers were in stage I (Table 3). This stage distribution is more advanced than reported in the literature. Self-selection by symptomatic individuals is a recognized source of confounding of prevalence results. Only 8 of the 27 (29.6%) of the prevalence cases and 7 out of 9 (78%) incidence cases reported symptoms

Preliminary Results, Molecular Airways Markers: Four markers are to be assayed in the sputum of Cohort participants:

Sputum Cytology: To date 263 individual subjects have had sputum specimens processed, stained and read by a pathologist (Table 5). Two (12.5%) of 16 cancer cases in the study showed sputum cytology indicative of cancer. For preliminary results see table (below).

hnRNP A2/B1 Overexpression: As outlined in the study protocol, ThinPrep monolayer slides are produced from methanol-preserved (PreservCyt) slurries of induced sputum.

At present, we are resolving several issues related to 703D4 immunoassay performance prior to immunostaining the Cohort specimens. We have been funded (Cancer Research Foundation of America) to identify the lung cancer epitope identified by NCI monoclonal antibody 703D4. Specificity of old and new lots of the 703D4 monoclonal are being compared by Western blot to 2 American and 2 Japanese antibodies against hnRNP. Epitope mapping of 703D4 has shown 3 hnRNP binding sites. Peptide oligomers made with these binding site sequences have been used as blocking peptides in immunostaining assays. Seven additional peptides that overlap the epitope have been made. An Elisa assay with 703D4 has identified the epitope within a single 12 amino acid peptide. We have purified the supernatant from clones deposited with ATCC by Dr. James Mulshine to derive a new monoclonal. This antibody is undergoing testing for clinical relevance and the antibody target is being sequenced.

Following the generation of the new monoclonal, slides will be stained (per protocol as follows: Following automated immunostaining (DAKO immunostainer) with the hnRNP A2/B1monoclonal antibody and alkaline phosphatase labeling (LSAB-II, DAKO), individual cells of interest (proplastic, metaplastic and atypical morphologies) are identified by a licensed cytotechnologist. Images of selected cells are acquired at 100 X (Nikon E800 equipped with Princeton Instruments cooled CCD) and quantified automatically for morphologic and densitometric parameters by a workstation running MetaMorph software (Universal Imaging Corp). If this image quantitation is successful, screening will be performed with fluorescent tags on our high throughput Laser Scanning Cytometer.

Loss of heterozygosity (LOH): Eighty-one alleles reported in the literature to be frequently lost in NSCLC or associated with the genes for transforming growth factor ß type II receptor (TBR II) or the downstream signaling SMADs 2 or 4 were examined to confirm their utility in a panel of microsatellite alteration (MA) markers for Cohort specimens. After establishing the PCR conditions (using ^{32}P end-labeling) for the primers at each allele, the primers were applied to archived (non-microdissected) DNA from 43 frozen paired tumor and normal samples. We evaluated microsatellite instability (i.e., shifts; MI) or loss of heterogygosity (LOH), reducing our final panel to 15 markers according to the frequency of these MA on both tumor tissue and sputum cell DNA templates. After blinded testing of 56 pre-neoplastic screening sputum specimens, lung cancer cases demonstrated LOH at two or more alleles significantly more often than controls. Nevertheless, using microsatellite markers to detect pre-clinical lung cancer in sputum is a challenge due to the large number of positives among cigarette smokers who have not developed lung cancer. While inactivating mutations in genes involved as "caretakers" of different DNA mismatch repair pathways are commonly observed in tumors, each individual may have a different set of defects in these check point genes limiting the screening application of the MA assay.

Promoter CpG Island Hypermethylation: Silencing of tumor suppressor genes (TSG) is one mechanism believed to underlie carcinogenesis. TSG silencing

may be accomplished through gene alteration (allelic loss or gene mutation). An epigenetic mechanism, promoter CpG island hypermethylation has also been shown to silence TSG transcription (Proc Natl Acad Sci USA, 1996; 93:9821-6). Panels of primers for hypermethylation have been recently published and are under study in our laboratory. We have established the conditions for assay of p16, O^6-MGMT, RAR-ß, and DAP-kinase promoter methylation in our laboratory. Four of seventeen (24%) frozen, paired (non-microdissected) DNA specimens demonstrate p16 promoter hypermethylation in our hands. To assure the quality of archived Cohort DNA, 5 Aliquots of DTT/EDTA/DMSO preserved Cohort sputum (1 cancer, 4 noncancers) have been sent to Dr. Adi Gazdar (Texas Southwestern) for assay of methylation of promoter of RASSF1, RAR- ß, p16, APC, E/H Cadherin. Preliminary results indicate the presence of satisfactorily preserved DNA at the promoter methylation sites in all specimens.

Lung Cancer and Ventilatory Obstruction: To investigate individual susceptibility to lung cancer, and potential mechanisms linking pulmonary obstruction to lung cancer, Dr. Jong Park will examine genetic polymorphisms in a case-control study of Cohort plasma specimens.

Each individual's genetic polymorphic profile will be examined with respect to a) the metabolic activation/detoxification of tobacco carcinogen [epoxide hydrolase (EH), glutathion-S-transferase P1 (GSTP1), GSTM1, cytochrome P4501A1 (CYP1A1), CYP1B1]; b) elastase-antielastase protein balance [neutrophil elastase (NE), α_1-antitrypsin deficiency allele (α_1-AD), matrix metalloproteinase1 (MMP1)]; and c) DNA repair [O^6-alkylguanine DNA alkyltransferase (AGT), human oxoguanine glycosylase1 (hOGG1), XRCC1].

Preliminary Results, Archive: Two thousand and twenty-two (2022) induced sputum specimens (includes annual repeats) have been prepared with dithiothreitol (DTT) and EDTA, washed in Hanks solution, spun, resuspended and divided into aliquots for freezing with 10%DMSO/90% FBS in liquid nitrogen. CYTYC Thin-prep slide preparations (for pap staining, immunostaining and storage), are to be made from CYTYC PreservCyt (methanol) slurries stored at 4°C. One thousand, nine hundred and ninety-three (1993) spontaneous specimens are also available as preserved slurries. One thousand, one hundred and forty-nine (1149) blood specimens have been processed; the buffy coats have been separated and stored in liquid nitrogen. Six hundred and forty-five (645) buffy coats and six hundred and sixty-three (663) induced sputum aliquots have been processed for DNA. Each specimen (except those in liquid nitrogen or −80 freezer) is bar coded, and computer linked to the database of registration, demographic, medical, smoking, occupational and nutritional history data on each participant.

Database and Lab Specimen Tracking System: Moffitt Cancer Control Research Computing has developed an Oracle database with a Web front-end to allow registration from multiple sites. This database houses the registration, demographic, medical, smoking, occupational and nutritional history data on each participant. Since

data entry is still forms-based, the data system was designed to provide easy, intelligent 'double' entry of data. The system has been programmed to provide data constraints, range and referential checks, and edit capability to keep the data clean. The data system provides tools for subject management (generate barcode labels, track unresolved data, report late forms/specimens, etc.). Finally, the relational database will easily provide data for specific queries and statistical analysis. This Research Specimen Tracking (RST) system has now been requested for application to the NCI-SPORE-Lung Cancer Biomarker and Chemoprevention Consortium (LCBCC) study.

Moffitt Cancer Control Research Computing also has developed a Laboratory Specimen Tracking System. This study generates a large number of specimens that must undergo multiple assays in several laboratories. The Laboratory Specimen Tracking System (LST) reads the 2-D specimen barcode to log the specimen into the laboratory. The LST has been programmed to assign each type of specimen a 'profile' that specifies what will happen to the specimen in the lab. A 'profile' consists of a number of steps such as: CheckIn/CheckOut, Assay specimen acceptability, Results Reporting and Archive. The LST is able to track the progress of the specimen and let the lab manager know what step the specimen is on, the specimen turnaround time in the lab, and the archive location of the specimen and its offspring including: Slides, Sputum Slurry Bottles, and Cryovials.

Key Research Accomplishments

- Developed an infrastructure to identify, accrue, screen and follow a non-diseased community-dwelling population at high risk for lung cancer.

- Developed procedures for collection and preservation of sputum specimens for new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of airways cytologic specimens suitable for evaluation of new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of white blood cells suitable to provide individual control specimens for DNA and RNA

- Developed a potency assay for MoAb 703D4 immunodetection of hnRNP A2/B1 protein expression.

- Identified a panel of 15 LOH markers for sputum lung cancer screening which identifies 84% of lung tumors.

- Completed the initial lung cancer screeing of 1151 middle-aged current and former smokers. Approximately 50% are found in stage I, a 3-fold greater frequency of stage I detection compared to the current clinical standard of no screening as reported by the Florida Cancer Registry.

Reportable Outcomes
Publications Related to this Study

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2. Toyooka S, Toyooka KO, Miyajima K, Reddy JL, Toyota M, Sathyanarayana UG, Padar A, **Tockman MS**, Lam S, Shivapurkar N, Gazdar AF. Epigenetic down-regulation of death-associated protein kinase in lung cancers. Clin Cancer Res. 2003; 9:3034-41

3. Zhukov TA, Johanson RA, Cantor AB, Clark RA, **Tockman MS**. Discovery of distinct protein profiles specific for lung tumors and pre-malignant lung lesions by SELDI mass spectrometry. Lung Cancer. 2003 ;40:267-79.

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7. Chirikos T, Hazelton T, **Tockman MS**, and Clark R. *Screening for Lung Cancer with CT: A Preliminary Cost-Effective Analysis.* CHEST 2002; May 121:1507-14.

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1. Zhukov TA, Johanson R, **Tockman MS**. *Discovery of distinct protein profiling specific for lung tumors and pre-malignant lung lesions by SELDI mass spectrometry.* Proceedings of the AACR, Volume 43, March 2002. Page 36, #184.

2. Zhukov TA, Erozan YS, **Tockman MS.** *Sentinel Cell for Lung Cancer.* 14th International Congress of Cytology, RAI Congress Centre, Amsterdam, The Netherlands. May 2001.

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6. **Tockman MS**, Saccomanno G, Michels R, Zhukov TA, Erozan Y, and Gupta P. *Sentinel Cell for Lung Cancer.* 6th SPORE Investigator's Workshop; July 1998.

Presentations Related to this Study

December 8-10, 1998   ***International Conference on Prevention and Early Diagnosis of Lung Cancer,*** *Johns Hopkins Lung Project* and *Immunocytochemical Screening for Lung Cancer.* University of Varese and University of Massachusetts Medical School, Varese, Italy.

February 12, 1999   ***ALCASE Workshop – Lung Cancer: A Revolution in Care,*** *Technology in Early Diagnosis of Lung Cancer.* Embassy Suites, Tampa, Florida

April 26, 1999   **1999 ALA/ATS International Conference Program,** *Early Sputum Marker for Lung Cancer (hnRNP).* San Diego Convention Center, San Diego, California

April 30, 1999   **Pharmacology Seminar Program,** *Detection and Immunostaining of the Lung Cancer Sentinel Cell.* University of Pittsburgh, Pennsylvania.

September 13, 1999   **Advanced Cancer Detection Center,** *External Advisory Committee.* Moffitt Cancer Center, Tampa, Florida

September 30th to   **The First International Conference On Screening for Lung,**
October 3, 1999   **Cancer,** Cornell University, New York

October 9-13, 1999   **Annual Congress of the European Respiratory Society,** *Dysregulation of the Cell Cycle in Lung Cancer.* Madrid, Spain

October 15-16, 1999   **Molecular Biomarkers Workshop,** Roy Castle Lung Cancer Foundation, Liverpool, England

October 26, 1999   **Screening of Lung Cancer Conference,** Gaithersburg, MD

October 31, 1999   **7th Annual Scientific Assembly of the American Association of Bronchology,** *New horizons in cytological based early detection in lung cancer.* Chicago, IL

February 9, 2000   **International Symposium on Early Detection of Lung Cancer,** *Molecular Screening Program: Past, Present, and Future.* Tel Aviv, Israel

February 27-29, 2000   **International Agency for Research on Cancer, Use of Biomarkers in Chemoprevention of Cancer,** *Lung Cancer: Intermediate Effect Markers.* Heidelberg, Germany

March 20, 2000   **Cahan Lectureship at Memorial-Sloan Kettering,** *Molecular Screening for Lung Cancer.* New York, NY

April 12, 2000   **Early Detection Research Network Site Visit at H. Lee Moffitt Cancer Center & Research Institute,** *Organization of BeDLAM.* Tampa, FL

June 16, 2000   **Wayne State University Cancer Conference,** *Sputum in 2000: Hypothetical Advantages, Practical Limitations, and Novel Approaches,* Detroit, MI

June 22, 2000   **Reducing Lung Cancer Mortality: Actions for the New Millenium,** *Sputum Based Detection of Preinvasive Lung Cancer,* Washington, DC

June 27, 2000   **Roy Castle Lung Cancer Foundation and H. Lee Moffitt Cancer Center, Quest for the Cure,** *Lung Cancer Screening and Early Detection: Spiral CT Scanning and Molecular Markers,* Tampa, FL

July 19, 2000   **H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,** *Epidemiology and Early Detection of Lung Cancer,* Coeur d'Alene, ID

July 19, 2000   **H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,** *The Management of Pre-Clinical Lung Cancer,* Coeur d'Alene, ID

September 12, 2000   **IASLC 9th World Conference on Lung Cancer,** *Cellular Targeting in the Molecular Diagnosis of Lung Cancer,* Tokyo, Japan

October 24, 2000   **66th International Scientific Assembly of the ACCP,** San Francisco, CA

ACCP Post Graduate Course, *Screening and Early Detection of Lung Cancer*

Meet the Professor, *Sputum Detection of Early Lung Cancer: Hypothetical Advantages, Practical Limitations, and Novel Approaches*

October 27, 2000   **Cornell CT Conference,** *Sputum Detection of Early Lung Cancer: A Compliment to Helical CT ,* New York, NY

March 7-8, 2001   **Lung Cancer Early Detection Workshop,** National Cancer Institute/ American Cancer Society, **"New Frontiers of Screening Science".** Rockville, MD

March 24-25, 2001   **Second Annual – A Practical Pulmonary Review for Primary Care Providers,** University of South Florida/Department of Veterans Affairs, James Haley, **"Early Recognition of Lung Cancer".** St. Pete Beach, FL

June 20-22, 2001   **Early Detection Research Network,** National Institute of Health/National Cancer Institute, *"Lung Cancer Screening Update"* and *"Industrial Partnership with EDRN",* Washington, DC

June26-July2, 2001   **Second International Lung Cancer Molecular Biomarkers Workshop "A European Strategy for Developing Lung Cancer**

Molecular and Clinical Diagnostics in High Risk Populations,
Roy Castle Lung Cancer Foundation, *"Sputum in 2001: Hypothetical Advantage Practical Limitations, and Novel Approaches"* and *"Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers"* Liverpool, England

August 7-12, 2001   ***3rd International Conference on Prevention & Early Detection of Lung Cancer,*** International Association for the Study of Lung Cancer, *"Cellular Approaches to Lung Cancer Detection"* and *"Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers".* Rejkjayik, Iceland

October 13-17, 2001   **EDRN Scientific Workshop,** Seattle, WA

October 26-29, 2001   **5th International Conference on Screening for Lung Cancer,** New York, NY

December 5-6, 2001   **NCI Grant Review Meeting RFA CA-02008 Chemoprevention of Tobacco-related Cancers in Former Smokers: Preclinical Studies,** Washington, DC

February 3-5, 2002   **EDRN Steering Committee,** Houston, TX

March 10-15, 2002   **New Frontiers in Cancer Detection & Diagnosis (EDRN/ Gordon Research Conference),** Ventura, CA

April 5-7, 2002   **6th International Conference on Screening for Lung Cancer,** Paris, France

June 13, 2002   **NIH Women Tobacco & Cancer Steering Committee,** Besthesda, MD

June 22, 2002   **Great Cancer Roundup: Lung Cancer Screening & Prevention Conference,** Los Angeles, CA

September 3-5, 2002   **6th EDRN Steering Committee,** Ann Arbor, MI

October 11-15, 2002   **Molecular Targets in Cancer Therapy,** St. Petersburg, FL

October 18-20, 2002   **7th International Conference on Screening for lung Cancer,** New York, NY

October 28-30, 2002   **1st International Lung Cancer Conference,** Beijing, China

Funding Received Based Upon Work Supported by this Award

1. "The Biomarker Development Laboratory at Moffitt" (NCI-CA 84973, M. Tockman, PI, 1st year/Total award $413,720/$1,903,827).
2. "Identification of the lung cancer epitope identified by the monoclonal antibody 703D4" (Cancer Research Foundation of America, M. Gruidl, PI, Total award $38,950)
3. J. Park, PI, NCI-EDRN, Total award $98,000.

Conclusion

The Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (DoD Cohort Study) has completed its desgined accrual and has submitted a report of the prevalence lung cancer screening experience for publication. This research question addresses the most common cause of cancer death and the only common cancer for which no screening is available. Second, the archive of radiographs, sputum and blood cell specimens provides an infrastructure for other investigators at Moffitt and across the nation. The recent award to Dr. Jong Park of an NCI Early Detection Research Network grant was based on the availability of the Cohort archive. Similarly, the collaboration with SPORE investigators at Texas Southwestern (Drs. Gazdar and Minna) and at Johns Hopkins (Drs. Baylin and Herman) are based upon the availability of Cohort specimens. The prospective design, innovative methods and careful execution of this study make it a valuable scientific contribution.

Table 1

Total Population Demographics

Subjects Enrolled (*N*=1151)	Obstructed	Unobstructed	P-Value
	(*n*=682)	(*n*=469)	
Age (y) +/- SD	62.0 +/- 8.3	57.6 +/- 8.0	P<0.0001
Sex (M/F)	414/268	270/199	
Average Pack Years (years) +/- SD	62.1 +/- 27.3	51.6 +/- 22.5	P<0.0001
Race No. (%):			
African American	24 (3.52)	13 (2.78)	
American Indian, Eskimo	3 (0.44)	*	
Asian or Pacific Islander	1 (0.15)	*	
Eastern Indian American	1 (0.15)	*	
White Hispanic	11 (1.62)	9 (1.9)	
White Non-Hispanic	639 (93.7)	446 (95.1)	
Other	3 (0.44)	1 (0.2)	
Average FEV1/FVC (%) +/- SD	57.5 +/- 10.7	77.3 +/- 4.4	P<0.0001

Table 2

Moffitt Cohort Study Results

	Study Design	Actual (as of 08/29/03)
Screened with Spirometry:	5000	3496
Eligible ~ screened with CT and Sputum:	1150 (23%)	1151 (33%)
Expected Positive Prevalence Screens:	230-460 (20-40%)	406 (35%)
Expected Cancers:	12-13 (1.1%)prevalence 12-13 annual incidence x 4	28 (2.4%) Prevalence 11 (0.76%) Incidence
Exp. Stage Distribution:	80% Stage 1	**Prevalent Cases:** Stage I: 14 (50.0%) Stage II: 2 (7.1%) Stage III: 3 (10.7%) Stage IV: 5 (17.9%) Limited SCLC: 2 (7.1%) Extensive SCLC: 1 (3.6%) Lymphoma: 1 (3.6%) **Incident Cases:** Stage I: 7 (63.6%) Stage II: 1 (9.1%) Stage IV: 1 (9.1%) Extensive SCLC: 1 (9.1%) Staging unknown: 1 (9.1%)

Table 3

Demographic Characteristics of Prevalent Lung Cancer Cases

Prevalent Lung Cancer Cases (*N*=28)	Obstructed (*n*=24)	Unobstructed (*n*=4)
Age (y) +/- SD	64.4 +/- 7.9	64.0 +/-10.2
Sex (M/F)	12/12	1/3
Average Pack Years (years) +/- SD	62.9 +/- 24.3	47.1 +/- 4.1
Race No. (%):		
African American	*	*
American Indian, Eskimo	*	*
Asian or Pacific Islander	*	*
Eastern Indian American	*	*
White Hispanic	1 (4.17)	*
White Non-Hispanic	22 (91.67)	4 (100)
Other	1 (4.17)	*
Average FEV1/FVC (%) +/- SD	60.2 +/- 10.5	72.3 +/- 0.2
Percent of Cancers in Population Screened:	3.5%	0.85%
Percent of Total Prevalent Cancers:	85.7%	14.8%
No. (% of cancers) [% of Screened] of Lung Cancer Cases in Stage:		
Stage I (NSCLC)	12 (50.0) [1.8]	2 (50) [0.43]
Stage II (NSCLC)	2 (8.3) [0.3]	*
Stage III (NSCLC)	3 (12.5) [0.44]	*
Stage IV (NSCLC)	5 (20.8) [0.73]	*
Limited Small Cell	1 (4.2) [0.15]	1 (25) [0.21]
Extensive Small Cell	*	1 (25) [0.21]
Lymphoma	1 (4.2) [0.15]	*

Table 4

Demographic Characteristics of Incident Lung Cancer Cases

Incident Lung Cancer Cases (N=11)	Obstructed (n=11)	Unobstructed (n=0)
Age (y) +/- SD	66.5+/- 6.9	*
Sex (M/F)	10/1	*
Average Pack Years (years) +/- SD	79.5 +/- 26.7	*
Race No. (%):		
African American	*	*
American Indian, Eskimo	1 (9.0)	*
Asian or Pacific Islander	*	*
Eastern Indian American	*	*
White Hispanic	*	*
White Non-Hispanic	10 (91.0)	*
Other	*	*
Average FEV1/FVC (%) +/- SD	52.4 +/- 11.1	*
Percent of Cancers in Population Screened:		
No. (% of incident cancers) of Lung Cancer		
Cases in Stage:		
Stage I (NSCLC)	7 (64)	*
Stage II (NSCLC)	1 (9)	*
Stage III (NSCLC)	*	*
Stage IV (NSCLC)	1 (9)	*
Extensive Small Cell	1 (9)	*

Table 5

Cohort Sputum Cytology Results

Prevalent Cancer Cases N=28	Induced Fresh Smear	Induced Thin Prep	Spontaneous Thin Prep
No Significant abnormality	7 (25%)	4 (14.3%)	9 (32.1%)
Regular metaplasia	7 (25%)	10 (35.7%)	5 (17.9%)
Mild Dysplasia	1 (3.6%)	1 (3.6%)	*
Moderate Dysplasia	*	1 (3.6%)	1 (3.6%)
Invasive Carcinoma, Squamous Cell	*	1 (3.6%)	*
Adenocarcinoma	1 (3.6%)	1 (3.6%)	1 (3.6%)
Unsatisfactory Specimens	6 (21.4%)	4 (14.3%)	6 (21.4%)
Results not available	5 (17.9%)	6 (21.4%)	6 (21.4%)

Prevalent Slides Read	Induced Fresh Smear	Induced Thin Prep	Spontaneous Thin Prep
No Significant abnormality	109 (40.4%)	61(22.8%)	112 (43%)
Regular metaplasia	96 (35.6%)	156 (58.4%)	106 (40.6%)
Mild Dysplasia	9 (3.3%)	11 (4.1%)	5 (1.9%)
Moderate Dysplasia	1 (0.37%)	2 (0.75%)	4 (1.5%)
Invasive Carcinoma, Squamous Cell	*	1 (0.375%)	*
Adenocarcinoma	1 (0.37%)	1 (0.375%)	1 (0.38%)
Unsatisfactory Specimens	53 (19.6%)	35 (13.1%)	33 (12.6%)
Results not available	881	884	890

First Annual Screening Slides	Induced Fresh Smear	Induced Thin Prep	Spontaneous Thin Prep
No Significant abnormality	18 (28%)	8 (12%)	12 (19%)
Regular metaplasia	37 (57%)	44 (67%)	44 (69%)
Mild Dysplasia		2 (3%)	1 (2%)
Moderate Dysplasia	*	*	*
Invasive Carcinoma, Squamous Cell	*	*	*
Adenocarcinoma	*	*	*
Unsatisfactory Specimens	10 (15%)	12 (18%)	7 (11%)

Second Annual Screening Slides	Induced Fresh Smear	Induced Thin Prep	Spontaneous Thin Prep
No Significant abnormality	2 (25%)	1 (12.5%)	2 (25%)
Regular metaplasia	5 (62.5%)	7 (87.5%)	2 (25%)
Mild Dysplasia	*	*	*
Moderate Dysplasia	*	*	*
Invasive Carcinoma, Squamous Cell	*	*	*
Adenocarcinoma	*	*	*
Unsatisfactory Specimens	1 (12.5%)	*	4 (50%)

Lung Cancer Screening with Computed Tomography: results of baseline (prevalence) screening

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Introduction

Lung cancer is the leading cause of cancer deaths for both men and women in the United States 1. The survival rates are dismal: 14% at five years and 7% at 10 years 2.

Survival is related to the stage at presentation. The five-year survival rate for patients with localized (node negative) tumors is over 50% 3'4 while the survival rate for stage IA (T1N0M0) disease is 60-80% 5'6'7. More than 50% of patients have distant metastases at diagnosis and only 25% of tumors are localized and potentially resectable for cure 8. Effective methods of early detection, therefore, might improve lung cancer survival and mortality rates.

Previous randomized, controlled trials of lung cancer screening with chest radiography alone or in combination with sputum cytology demonstrated no mortality reduction benefit to screened groups 9'10'11'12'13'14. Recently, interest in lung cancer screening has been revived using low-dose, single breath, helical computed tomography (CT)15'16'17'18'19'20. These recent studies have shown that screening with CT can detect lung cancers at smaller size, and earlier stage, than chest radiography and current clinical practice. However, to date, mortality reduction benefit from screening CT has not been demonstrated.

We are conducting a prospective, longitudinal, single-arm cohort screening trial, with the hypothesis that screening with CT and sputum molecular markers will increase the proportion of stage I cancers to over 60% of total cohort lung cancers. The study design includes one baseline (prevalence) screen, and four subsequent annual repeat (incidence) screening rounds. The purpose of this paper is to report our results of the baseline, or prevalence, round of screening for lung cancer with CT.

Material and Methods

Our university Institutional Review Board (IRB) and the IRB of the Department of Defense (DOD) approved the study protocol. Participants were enrolled after meeting eligibility requirements and giving written informed consent.

Eligible subjects were asymptomatic women and men 45 years of age or older. Participants had to be current or former cigarette smokers, with a history of cigarette smoking of at least 30 pack-years. Ineligible were those with a history of any cancer other than non-melanoma skin cancer.

All participants agreed to undergo spirometry, with measurement of forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC), a venous blood specimen, induced sputum collection plus a 3-day mailer for spontaneously produced sputum, and a CT scan at each annual visit.

Screening CT examinations were performed using single channel and multichannel helical (Siemens software) scanners. Subjects were scanned with a table movement of 20 mm/sec, a 2:1 pitch, 120 kVp, mA ranging from 20 - 80, and reconstructed image thickness of 10mm. Screening scans were done with a single breath-hold acquisition without intravenous contrast medium.

All screening CT images were interpreted at a PACS (picture/archive/ communication system workstation) (Siemens MagicView 1000) independently by one of three investigator radiologists. Images were viewed in both lung (W 2000, L −600) and soft tissue (W 350, L 20) window/level settings. The location and size of any non-calcified nodule or opacity, and any additional findings were reported.

The criteria for an abnormal screening CT examination included any noncalcified pulmonary or hilar mass, nodule or opacity of any size that could represent lung cancer.

Written diagnostic management recommendations for CT findings were made in each case to the attending physician based on a management algorithm for indeterminate lung nodules or opacities, similar to those used in other cohort CT screening trials [15,16,17,18,19,20] (Figure 1).

Discrete variables were compared with Fisher's Exact or Chi Square Tests. Means were compared with the Student's t test and rates were modeled as Poisson variables and compared using a normal approximation.

Results

From December 4, 1998 to October 10, 2002, 1,151 participants were enrolled and underwent the baseline prevalence CT scan.

The characteristics of the subjects enrolled in our trial are summarized in Table 1. Overall, 59% of subjects were male and 41% female, and the mean age was 60 years. The mean duration of smoking was 58 pack-years.

In the cohort, 406 (35%) of the subjects had an abnormal baseline (prevalence) CT, and all received subsequent diagnostic evaluation according to our protocol. Diagnostic evaluation was limited to periodic CT imaging according to protocol (Figure 1) in 374 participants. Positron emission tomography (PET) scans were performed in 15 patients. Percutaneous needle biopsy was performed in 11 patients. Bronchoscopy and/or mediastinoscopy were performed in 18 patients (several participants had multiple diagnostic procedures).

Screening CT detected 28 neoplasms (2.4% of 1151 subjects), including one non-Hodgkin lymphoma and two small cell lung carcinomas (SCLC). There were 25 cases of non-small cell lung cancer (NSCLC) detected in the prevalence screen (NSCLC prevalence rate 2.2% of 1151 subjects), and all had complete staging and pathological review.

Surgical pulmonary resection of NSCLC was performed in 20 participants (pneumonectomy in 1, pulmonary lobectomy in 12, segmentectomy in 7 and mediastinal lymphadenectomy in all 20). NSCLC was diagnosed and staged without surgery in 5 patients with stage IV disease. Surgery yielded a benign diagnosis in 4 patients: 2 benign non-caseating granulomas, 1 aspergillus granuloma, and 1 benign intra-parenchymal lymph node.

The characteristics of the patients with NSCLC are summarized in Table 2. Twenty-two of the 25 (88%) NSCLC cases occurred in subjects with pulmonary obstruction (FEV1/FVC $\leq$ 70%). Obstructed subjects sustained a 5-fold elevation in prevalence lung cancer rates compared with unobstructed subjects ($\chi^2 = 8.75$, p = 0.003). The prevalence cancer rate in obstructed subjects was 3.2%; in unobstructed subjects, 0.64%. Even though 59% of the cohort subjects were male, NSCLC was detected more often in women (52% of NSCLC) than in men (p = 0.24).

The size, stage, and cell type distributions of the detected cancers are summarized in Table 3. There were 14 stage I cancers (56%), of which 12 were stage IA. The prevalence screening detected 11 adenocarcinomas (44%), 6 bronchoalveolar carcinomas (24%), 3 adenocarcinoma with bronchoalveolar features (12%), 2 squamous cell carcinomas (8%), and 3 large cell carcinomas.

The mean tumor size (T size) of detected NSCLC was 21 mm (9-60 mm). Nineteen of the 25 cases (76%) of NSCLC were T1 cancers ($\leq$ 30 mm.); the mean size of T1 cancers was 16 mm (9-30 mm).

We compared our prevalence results to prevalence CT screening results from 6 other published trials (Table 4). Our rate of abnormal prevalence screening CT scans (35%) was between the lowest rate of 5% and the highest rate of 51% (a 10-fold range)[21]. Our prevalence NSCLC detection rate (2.2%) was 2nd highest of all the trials (highest, 2.7%; lowest, 0.4%). The sizes of detected cancers in our study were slightly larger than those detected in the other reported studies. Over all the other studies, 24% of the detected cancers were $\leq$ 10 mm. in greatest diameter, while 23% were greater than 20 mm. in diameter. In our trial, similar outcomes were 20% and 36% respectively. Similarly, over all the other studies, 83% of detected cancers were stage I, while none were stage IV; our results for stage I and IV were 56% and 20% respectively ($\chi^2 = 29.8, \pi < 0.0001$).

Discussion

Our results confirm that CT can identify small and early-stage lung cancers. Most (56%) of the non-small cell lung cancers detected by computed tomography were stage I at diagnosis. This approaches our stated hypothesis that CT screening would increase in the proportion of stage I cancers detected by screening to 60% of total cohort lung cancers. We must await the annual repeat (incidence) experience to determine if this rate of early stage diagnosis is maintained and accompanied by a lower frequency of advanced stage cancers (i.e., a stage shift). At least one economic model[22] suggests that if detection of 60% of lung cancer in stage I were accompanied by a reduction in the lung cancer mortality rate, that screening would be cost-effective.

False positive screening CT examinations are a considerable concern. In our prevalence round of screening, the positive predictive value for interpretation (PPV_1)[23] of an abnormal screening CT for neoplasm was 6.9% (PPV_1 = true positive cases $\div$ abnormal screening exams = 28/406), and for NSCLC was 6.2% (25/406). This is similar to the expected PPV_1 for breast cancer screening: 5-10%[23,24], an accepted and widespread screening practice. Although our "recall rate"[24] for CT screening (35%) is higher than that expected for mammography screening (<10%[23]), the prevalence lung cancer yield is greater than the breast cancer yield, so the predictive values are similar. The range of PPV_1 values for the existing cohort screening trials is 2.3% - 11.6% (Table 4). Our positive predictive value for biopsy (PPV_2) was 88% (PPV_2 = cases of neoplasm at biopsy $\div$ all biopsy cases = 28/32), much higher than that expected for breast cancer screening: 20-40%[23,24].

Our results differ from the prevalence CT screening results of the other trials. At baseline screening, we observed fewer stage I cancers and more stage IV cancers than those reported in the other baseline screening trials. In our series, 76% (19/25) of screening detected cancers were T1 lesions, yet only 56% (14/25) were stage I. Moreover, the mean sizes of our stage III and IV cancers were small (20 and 18 mm. respectively). One of two stage III cancers and 2 of 5 stage IV cancers were less than 10 mm. in greatest diameter.

Therefore, 26% (5/19) of our screening-detected T1 cancers had regional or distant metastasis at time of detection. This is similar to other published reports of the

stage distribution of small lung cancers, in which metastases were present in 17%-40% of T1 lung cancers 25,26,27 and 18% of cancers ≤ 10 mm. 28

Moreover, when T1 cancers are stratified by size, there is no correlation between tumor size and survival 29. Size of tumor alone therefore may not be an adequate measure of either biologic activity, or probability of regional and distant metastatic spread. There is currently limited data about survival rates of screening-detected T1 cancers.

The major hypothesis of CT screening is that cancer stage distribution depends primarily on tumor size at detection; i.e., detecting smaller tumors will result in a greater proportion of Stage I disease. We have observed that most (56%) of the non-small cell lung cancers detected by computed tomography were stage I at diagnosis. But the proportion of advanced stage cancers associated with T1 lesions suggests that small tumor size is only one component of early stage disease, casting some doubt that this crucial hypothesis is complete. Consideration of metastatic potential may be as important as lesion size for successful screening. This hypothesis would require the testing of a complementary biomarker of metastatic potential during trials of helical CT screening. Ongoing trials of lung cancer screening which combine both helical CT and biomarker collection offer an opportunity to test this hypothesis.

A multi-center, randomized trial has begun in the United States: the National Lung Screening Trial (NLST) funded by the National Cancer Institute (NCI) 30, 31, that compares screening with CT to screening with chest radiography. The enrollment criteria are women and men smokers or former smokers, ages 55 – 74 years and ≥ 30 pack-years of smoking history. The NLST measurement endpoints are mortality, quality of life and cost-effectiveness. Specimens of blood, sputum, and urine collected during the NLST may determine whether evaluation of lung cancer biological behavior is a necessary component of successful screening.

TABLE 1: Characteristics of subjects in prevalence (baseline) screening round

	Total Cohort	Obstructed * Subjects	Unobstructed ** Subjects
Age (years) +/- SD	60.2 +/- 8.4	62.0[1] +/- 8.3	57.6 +/- 8.0
Sex (male/female)	684 / 467	414 / 268	270 / 199
Mean pack-years smoking history +/- SD	57.9 +/- 26.0	62.1[2] +/- 27.3	51.6 +/- 22.5
Mean FEV1/FVC +/- SD	65.5 +/- 13.0	57.5[3] +/- 10.7	77.3 +/- 4.4

* Obstructed: $FEV1 / FVC \leq 70\%$
** Unobstructed: $FEV1 / FVC > 70\%$

SD = standard deviation
FVC = forced vital capacity
FEV1 = forced expiratory volume in 1 second

[1] Obstructed subjects are significantly older, $t=8.97$, $p < 0.0001$

[2] Obstructed subjects have a sig. greater smoking history, $t = 6.88$, $p < 0.0001$

[3] Obstructed subjects have a sig. lower FEV_1/FVC, $t = 37.9$, $p < 0.0001$

TABLE 2: Characteristics of patients with NCLC detected in prevalence (baseline) screening round

	Total Cohort	Obstructed * Patients	Unobstructed ** Patients
NSCLC (number)	25	22	3
Prevalence Rate NSCLC (%)	2.2%	3.2%[1]	0.64%
Age (years) +/- SD	64.0 +/- 8.4	64.5[2] +/- 8.2	59.0 +/- 12.7
Sex (male - female)	12 - 13	12[3] - 10	0 - 3
Mean pack-years smoking history +/- SD	61.0 +/- 24.5	62.2[4] +/- 25.2	47.8 +/- 6.7
Mean FEV1/FVC +/- SD	60.5 +/- 10.8	59.5[5] +/- 10.7	71.6 +/- 0.3

* Obstructed: FEV1 / FVC $\leq$ 70%
** Unobstructed: FEV1 / FVC > 70%

NSCLC = non-small cell lung cancer
SD = standard deviation
FVC = forced vital capacity
FEV1 = forced expiratory volume in 1 second

[1] Lung cancer prevalence is sig. greater among the obstructed, $\chi^2 = 8.75$, $p = 0.003$
[2] Obstructed patients are significantly older, $t = 8.9$, $p < 0.0001$
[3] Lung cancer is sig. assoc. with obstruction among males, $\chi^2 = 7.96$, $p = 0.005$
[4] Obstructed patients have a sig. greater smoking history, $t = 12.1$, $p < 0.0001$
[5] Obstructed patients have a sig. lower FEV_1/FVC, $t = 24.5$, $p < 0.0001$

TABLE 3: Characteristics of NSCLC cases detected in prevalence (baseline) screening round

	Number	%	Mean size (mm)	Range in size (mm)
NSCLC – all	25		21	9-60
NSCLC - T1 tumors (<= 30 mm.)	19	76%	16	9-30
Stage (number and % of total)				
IA	12	48%	16	10-26
IB	2	8%	35	
II	2	8%	40	24-60
III	4	16%	20	9-30
IV	5	20%	18	9-40
Cell Types of NSCLC				
Adenocarcinoma	11	44%	21	9-40
Adenocarcinoma with bronchoalveolar features	3	12%		
Bronchoalveolar carcinoma	6	24%	13	10-19
Squamous cell carcinoma	2	8%	43	26-60
Large cell undifferentiated	3	12%	19	16-24

NSCLC = non-small cell lung cancer　　　　　**mm = millimeters**

% = percentage

TABLE 4: Prevalence CT screening results from 7 clinical trials

Trial	A	(15)	B	(16)	C	(17)	D	(18)	E	(19)	F	(20)	G		Total (A-G)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Minimum age (years)	60		40		40		40		50		40		45			
Minimum smoking (pack-years)	10		0		0		0		20		20		30			
Subjects screened	1,000		5,483		7,956		1,611		1,520		817		1,151		19,538	
Abnormal screening CT	233	23%	279	5%	541	7%	186	12%	782	51%	350	43%	406	35%	2,777	14%
NSCLC PPV	27	11.6	23	8.2	37	6.8	14	7.5	18	2.3	12	3.4	25	6.2	155	5.7
Prevalence NSCLC		2.7%		0.4%		0.5%		0.9%		1.2%		1.5%		2.1%		0.8%
Tumor size (mean - mm)	15		15		17		20		17		NA		21			
NSCLC stage													*			
1A	22	81%	21	91%	29	78%	10	71%	12	67%	6	50%	12	48%	112	72%
1B	1	4%	2	9%	3	8%	1	7%	1	6%	1	8%	2	8%	11	7%
2	1	4%	0	0%	4	11%	0	0%	3	17%	3	25%	2	8%	13	8%
3	3	11%	0	0%	1	3%	3	21%	2	11%	2	17%	4	16%	15	10%
4	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	5	20%	5	3%
Total	27		23		37		14		18		12		25		156	

Cohort Trial References
A Henschke, et. al. (15)
B Sone, et. al. (16)
C Nawa, et. al. (17)
D Sobue, et. al. (18)
E Swensen, et. al. (19)
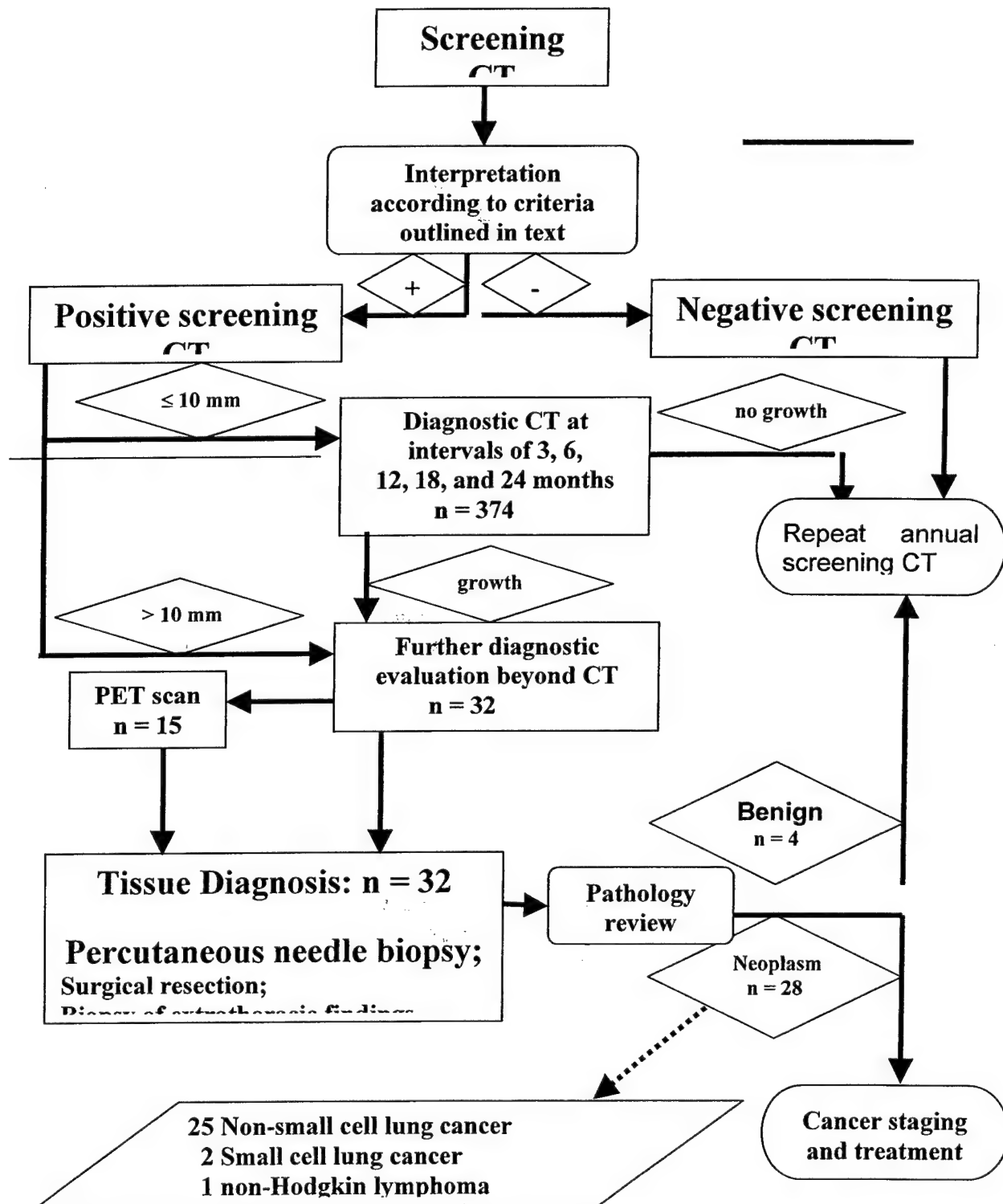F Diederich, et. al. (20)
G this trial

NSCLC = non-small cell lung cancer   mm = millimeters   n = number   % = percentage

PPV = positive predictive value

* The NSCLC stage distribution of this trial is significantly different from the aggregate of other trials, $\chi^2 = 29.8$, $p < 0.0001$

Figure 1: Diagnostic evaluation algorithm for abnormal prevalence   screening CT

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Appendix B

The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers

Jeffrey P. Krischer, Ph.D.

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Development of the Moffitt Cancer Network
Principal Investigator: Jeffrey P. Krischer, Ph.D.

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INTRODUCTION:

The Moffitt Cancer Network's (MCN) goal is to provide up-to-date oncology related information, resources, and education to oncology health care providers and researchers for the prevention and cure of cancer. Consistent with the aims of the Advanced Cancer Detection Center, the MCN provides access to educational programming, cancer control and clinical protocols, and a mechanism to exchange patient focused information leading to the improved detection and treatment of cancer. The MCN is health care provider focused and complements an array of existing public/lay information sources available elsewhere. It is built around the concept that oncology expertise is geographically centralized, multidisciplinary in nature and of limited availability. The MCN addresses these constraints by increasing availability through a World Wide Web-based design that enables wide access from many geographic locales. The objectives of this project are to:

Collect and organize cancer information to provide educational content to physicians and other health care providers,

Develop and implement software to encode video and audio to enable viewing over the Internet at a range of speeds (bandwidths),

Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation,

Design and create a web page to permit easy sorting, searching and selection of educational programming,

Design and create a web page to deliver physician referral information that includes submission of an electronic case record consisting of text and imaging data, and

Provide access to case conferencing from remote locations using easily available audio/video to the desktop.

BODY:

Task 1.   Collect and organize cancer information to provide educational content to physicians and other health care providers. (Months 1-60).

A schedule of events is determined in coordination with the Moffitt Office of Conference Planning, the Moffitt Multimedia Educational Resources Center, the USF Department of Education, the USF Department of Continuing Medical Education and independent researchers wishing to present. These events include: Grand Rounds, the monthly meeting of the Cancer Control Research Interest Group (CCRIG), Tech Topics (for medical information technology staff), a number of national and local oncology conferences, as well as a number of JCAHO requirements for in-service education for nurses, physicians, and other hospital staff.

The MCN currently has 567 presentations in its library, an increase of 71 from the previous year. Additionally, 16 conferences sponsored by USF and Moffitt are also currently available online.

Schedule videographer coverage of grand rounds and research conferences.
The Network Coordinator, in cooperation with Moffitt Department of Education and the Moffitt Multimedia Educational Research Center, compiles a schedule of for credit events. This schedule is used to determine the scheduling needs of the MCN videographer. The MCN videographer provides audio and video capture of these events digitally and to 90-minute DVCAM (Digital Video Camera) tapes when appropriate.

In late 2002 capture for the MCN migrated to 99% tapeless for routine presentations. Some special events and custom designed instruction are still recorded initially to tape and then digitized for editing. Whenever possible MCN captures presentations electronically without the use of videotape.

Coordinate notification of nursing, pharmacy and other health care providers continuing education presentations.
The Moffitt Department of Education notifies the MCN and all relevant clinical staff of all continuing education presentations and obtains a release from all speakers that permits the distribution of their respective presentation by the MCN. Notifications to clinical staff are multi-modal consisting of e-mails, web postings, and paper fliers. Notification to MCN and other staff required for recording of events is 100% electronic.

Organize the videotaping of faculty scientific presentations for national oncology conferences.
The notification and videotaping of national oncology conferences is scheduled in accordance with the system mentioned above, developed in coordination with the MCN and the Moffitt Education Department. A number of conferences have been added to the MCN library. These presentations are digitized and are made available on the MCN website. The presentations acquired by this activity are codified by a medical librarian, searchable by subject and grouped by their respective conference title.

Coordinate with the Department of Education notification and scheduling of relevant conferences.
The Moffitt Department of Education notifies the MCN of all relevant conferences and the MCN videographer is scheduled in accordance with the videotaping needs of each conference.

Task 2.   Develop and implement software to encode video and audio to enable viewing over the Internet in a range of speeds (bandwidths). (Months 1-60)

Explore the application of the Tag development software to support multiple video connections and the impact on network bandwidth.
The MCN has developed a process of digitizing presentations using the Digital Renaissance Tag Composer. Through this process MCN is able to stream presenter's slides and audio simultaneously by using a Synchronized Multimedia Integration Language (SMIL) script file. MCN originally encoded presentation for distribution over ISDN speeds of 128k and modem speeds of 56k. The encoding process used previously created two-network streaming formats, one for ISDN speed connections at 128 kilobytes per second and a second format for current modem technology speeds of 56 kilobytes per second or less. Using the Real media server software, users linking to a presentation acquire the format (streaming speed) appropriate for their connection bandwidth. The server and the user's player handle this process automatically.

Late in August 2000 MCN determined that the ISDN format was redundant, as it did not offer any significant improvement over the modem format due to the low frame rate of the presentations being developed (sometimes as low as one frame for every three minutes), and MCN has discontinued the encoding an ISDN bit rate media file and thus lowering the production time.

In July of 2000 MCN began to explore the use of the Microsoft Media suite of tools for development of online course content. Microsoft Media provides significant advantages in bandwidth reduction, production and administration time, and potential audience. The MCN has since migrated all processes to Microsoft Media. Windows Media supports a process called Multiple Bit Rate (MBR) video. Put simply, MBR video allows MCN to create a presentation geared toward either low (those users below 128k) or high (those users above 128k) bandwidth. The software determines the minimum speed required by the presentation to stream then negotiates between the client computer (the user) and the server the most bandwidth conserving connection. Using MBR video we are able to stream presentations at 28-32k which previously required 56k+ using Real media. In March of 2000 MCN began the process of converting all assets previously developed in Real media to the Microsoft Media format to better serve our users.

In August of 2001 MCN completed the conversion of all assets to Microsoft Media and began using Windows Media version 7, this provided significant quality improvements over Windows Media version 6.4 while reducing bandwidth requirements.

In 2002 the MCN began using Microsoft Media 8, which provided higher quality at a lower bandwidth than Window Media 7. Compatibility issues between Windows Media 8 and older operating systems forced us to revert back to Windows Media 7. All presentations recorded in Windows Media 8 were re-encoded as Windows Media 7. Since our migration back to Windows Media 7, Microsoft has released Windows Media 9 and stopped supporting Windows Media 8. In mid-2004 MCN will evaluate capturing all new assets in Windows Media 9 or a more recent Windows Media version if one is available and proves backwards compatible.

Evaluate alternative connectivity models, including cable modem connections or access to cable networks as a means to enhance distribution of educational content.

The MCN has evaluated multiple alternative connectivity models, including cable modems, ISDN, ADSL, and traditional T1 & T3 service lines. We have found that cable modems are an excellent method of distributing educational content. Cable modems and ADSL provide a low cost, high bandwidth alternative for the user. This allows educational content to become more dynamic and interactive increasing the quality and effectiveness of the educational activity.

In late 2002 and during 2003 MCN tested these connectivity models for various bandwidth levels of synchronous conventional videoconferencing. Findings show that within the controlled environment of the state of Florida University Internet connectivity backbone (also connected to Internet2) bandwidths above 256k were capable and exhibited time delays associated with conventional point to point ISDN based conferencing. Tests using TimeWarner's RoadRunner cable modem service in Tampa, Fl proved successful up to 384k. It should be noted however RoadRunner Tampa has a direct connection to the University of South Florida backbone. Tests using high speed DSL (768k) from Arizona to Tampa and Tampa to Tampa where successful and showed no significant transmission quality loss.

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Synchronous videoconferencing below 256k was widely successful on most mediums with the exception of the dial-up POTS (Plain Old Telephone System). Both conventional videoconferencing and more recent "Internet only" technologies where explored. Outside of a controlled environment, but with high speed connections (DSL, Cable Modem) low bandwidth videoconferencing is extremely successful and shows expected quality. It should be noted that delay previously associated with Internet and low bandwidth videoconferencing proved not to be significant. Low bandwidth videoconferencing shows to be a viable medium for dissemination of educational content as well as can be used to enchance collaboration and increase productivity between geographically disparate groups.

Evaluate the Internet 2 as to its availability to sustain the necessary bandwidth for the Moffitt Cancer Network.
The MCN has evaluated Internet 2 and found it is ideal mechanism for transporting images, streaming video to and conducting case conferences with other researchers and physicians. Internet 2 is highly effective and we will continue to utilize it when possible.

Resolve firewall and security issues to provide secure communication for clinical data as well as to adequately deal with subscriber/user requirements for security to permit desktop access.
A firewall has been put in place to ensure secure communications for clinical data and to address user security issues. Moffitt IT, in coordination with the MCN has developed a firewall policy relating to streaming media and videoconferencing.

The Moffitt Cancer Center uses key fob technology in conjunction with a secure ID for access to information through the firewall.

Uniform Resource Locator based on specific one-time virtual names.
All prerecorded media is encrypted when necessary and is assigned a unique access requirement for specific use. Users have no direct access to media assets and are provided a virtual link to the assets by a database driven web front end. Additional security methods are still being researched and firewall security is a priority.

Expand the number of Authorized users to the Moffitt Cancer Network.
Expansion of authorized users is critical to the digital convergence with MCN's ongoing research and development. We are now capable of delivering "On-demand", encrypted, and live media to desktops both user specific and publicly when appropriate. In addition, with the recent addition of continuing credit hours for nursing, we have opened a huge medical audience for MCN. It should be noted that there is no requirement to register or become authorized in order to watch most presentations available on the MCN.

Authorized users increased from 2 to 16 in the year 2000, an increase of 800%.

In mid 2001 a distinction was made between "authorized" and "registered" users. Authorized users are groups of predetermined people who are authorized to view a particular type of content. Registered users are either authorized users who have taken the time to register or non-authorized users who have registered for CME purposes. Authorized, registered users (previously referred to as just "authorized" users) increased from 16 to 68, an increase of 425%, in the year 2001.

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Due to recent outreach programs our user base continues to grow. In 2001 the number of authorized, registered users increased from 68-90, an increase of 32%. The number of fully registered users continues to rise at a steady rate. New programs with Moffitt affiliate hospitals established in 2001 generated even greater numbers of authorized, registered users.

Registered, authorized users increased from 90 to 237 in 2002.

Registered, authorized users increased from 237 to 384 in 2003. The number of authorized or registered users reflects only a segment of the utilization of the Moffitt Cancer Network. The overall usage statistics are a more valuable statistic to determine utilization. The statistics (below) show a regular progression in utilization over the past year of the Moffitt Cancer Network. The statistics are separated into internal (users internal to Moffitt Cancer Center) and external (those accessing via the internet). The combined value displays the number of presentations watched and the average number of presentations watched per user. Important to note is the number of sessions (visits) and number of presentations watched per month. The statistics show a regular increase from month to month in site utilization.

MCN Statistics
2002 July to 2003 June
Web Stats

	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03	Jun-03	Total
Internal													
Sessions	709	1035	704	689	343	496	325	287	662	374	356	327	6307
Hits	5287	8228	5771	4958	2899	3602	2546	2398	4633	3194	3161	2214	48891
H/S Ratio	7.457	7.950	8.197	7.196	8.452	7.262	7.834	8.355	6.998	8.540	8.879	6.771	7.824
Unique Vis	415	620	398	446	186	339	196	146	400	188	183	200	310
S/U Ratio	1.708	1.669	1.769	1.545	1.844	1.463	1.658	1.966	1.655	1.989	1.945	1.635	1.737
External													
Sessions	1771	2253	2180	1977	2027	1934	2513	2119	2458	2210	3051	2048	26541
Hits	6020	10086	10891	13497	11646	12473	12321	11862	13598	11345	13005	9521	136265
H/S Ratio	3.399	4.477	0.606	0.652	0.703	0.757	0.815	0.878	0.878	0.945	1.013	1.085	1.351
Unique Vis	887	1129	1197	1116	968	895	1043	1005	1007	1135	1317	1116	1068
S/U Ratio	1.997	1.996	0.307	0.331	0.356	0.384	0.413	0.445	0.445	0.479	0.513	0.550	0.685
Combined													
Sessions	2480	3288	2884	2666	2370	2430	2838	2406	3120	2584	3407	2375	32848
Hits	11307	18314	16662	18455	14545	16075	14867	14260	18231	14539	16166	11735	185156
H/S Ratio	4.56	5.57	5.78	6.92	6.14	6.62	5.24	5.93	5.84	5.63	4.74	4.94	5.66
Unique Vis	1302	1749	1595	1562	1154	1234	1239	1151	1407	1323	1500	1316	1378
S/U Ratio	1.90	1.88	1.81	1.71	2.05	1.97	2.29	2.09	2.22	1.95	2.27	1.80	2.00
Pres Watch	401	674	**650**	**460**	**296**	**365**	**369**	**307**	**467**	**293**	**356**	**252**	4890
P/U	0.31	0.39	0.41	0.29	0.26	0.30	0.30	0.27	0.33	0.22	0.24	0.19	0.29

H/S Ratio represents amount of time user spends on site per visit

S/U Ratio represents the average number of times users return

P/U Ratio represents number of videos the average user watched that month

Task 3. Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation. (Months 1-60).

Arrange for automated notification of Department of Education staff for each new presentation selected for the Moffitt Cancer Network.
USF Continuing Professional and Moffitt Conference Planning are notified of each new for-credit presentation selected. Upon successful completion of a CME activity, all relevant information is first stored to our database then is automatically transmitted to USF Continuing Education. The electronic submittal of credit information creates a paperless environment for USF, Moffitt Conference Planning, and MCN. The user is then forwarded to a PDF certificate of completion unique to the individual and activity completed. This further reduces the paper requirement on USF.

Establish ongoing procedures to obtain releases, objectives and CME questions to implement to permit encoding of presentations and inclusion onto the Moffitt Cancer Network.
Presenters sign a release to rebroadcast prior to the videotaping of their presentation. The Moffitt Department of Education works closely with the presenter and the MCN to establish objectives, determine appropriate CME questions and evaluate the overall quality of the educational content of the respective presentation. Upon the completion of this work, all information is passed to the appropriate staff for inclusion into the MCN website for delivery to the user.

Create documentation and procedures to collect appropriate demographics on individuals desiring CME and implement electronic automated notification of our Continuing Education Office to authorize and verify CMEs earned.
Appropriate demographic information is collected from all individuals wishing to receive CME credit for physicians or nurses contact hours. Upon completion of a CME credit or contact hours, the MCN staff is electronically notified. The results of the activity are graded electronically and the information is forwarded to the USF Education Department if a CME credit or contact hour was in fact earned.

In early 2001 MCN developed a process whereby all relevant information pertaining to the educational activity and credit received is transmitted via an encrypted data string directly into the USF Continuing Professional Education certificate-processing cue upon satisfactory completion of credit requirements. This eliminates a number of steps while reducing the probability of error. MCN currently uses a redundant system whereby USF Continuing Education records are audited periodically against MCN records to ensure proper certificate issuance.

Automatically link the Cancer Library to the acquisition process so that they are aware of new acquisitions and receive opportunities to extract key words for indexing, sorting and searching.
Upon the completion of the digitization of a presentation, the digitized presentation is forwarded to the Cancer Center Librarian for review. The Cancer Center Librarian extracts key words used for indexing, sorting and searching presentations on the MCN website. These keywords are added to the MCN website database for each respective presentation.

In late 2002 this became an automated process whereby the cancer center librarian is automatically notified when an event is added to the MCN. The librarian may then go to a keyword management system to enter data. The keyword management system is sortable and searchable and allows the library to get a snapshot of the state of the keyword database by seeing which presentations have been keyworded and which have not.

Extend the CME process to include CEUs for nursing and pharmacy.
The MCN currently offers CME credit for physicians as well as contact hours for nursing continuing professional education (CEU). The certifications are provided in cooperation with the USF College of Medicine and Nursing, respectively. We are continuing to explore the applicability of the content to other healthcare providers, such as pharmacists, and the requirements to offer continuing education credits.

In late 2002, the MCN began to issue Pathology certifications that have been highly utilized.

Expand the educational content offerings to include mandatory requirements for risk analysis, HIV, infection control, etc.

The MCN has expanded the educational offerings to include a number of JCAHO requirements for nurses, physicians and staff. These offerings are available internally to all personnel via the Moffitt Cancer Center Intranet. Major mandatory education such as Domestic Violence, HIV/AIDS, and Bioterrorism are available to external users as well.

MCN is now in the process of making available educational material regarding open clinical trials in addition to the topics mentioned above.

Task 4.   Design and create a web page to permit easy sorting, searching and selection of educational programming. (Months 1-24)

Organize educational content along primary audience lines and develop a key word searching algorithm to subset for presentations.

An algorithm has been developed allowing keyword searching. The keywords are assigned during the review of the presentation by the cancer center library. A new algorithm was developed in 2001 allowing a more efficient search. The MCN website provides chronological ascending/descending, keyword search, search within results, and presenter last name, first name searches.

Implement a database for key words according to a standard nomenclature, utilizing NLM MeSH headings, cancer site, etc.

A keyword database has been created and is used by the MCN website for searching. The keywords are determined by the Cancer Center Librarian prior to the addition of a presentation to the MCN. The keywords are based on NLM MeSH standards.

In late 2001 the MCN began to track user searches to gain a better understanding of how users search the website. The information gathered has led us to add not only MESH keywords, but layperson keywords as well.

Expand implementation of Active Server Page (ASP) extensions to the multimedia hypertext (HTML) by adding onto the 'back-end' of the Web application i.) procedural language scripting and ii.) the ability to exchange information with a fully functioning database.

ASP has been used throughout the site to produce dynamic, database driven web pages. ASP is used in all areas of the site to set procedural paths, increase security and generate dynamic content from the MCN databases.

Expand and refine the JET database to incorporate user defined search phrases that are located within a variety of fields associated with the database, including a textual 'objectives' section, MeSH headings, cancer site, canned search categories, etc.

The MCN has increased the capability of the Jet database to allow user defined search phrases. These phrases search for matches in the textual 'objectives' section, MeSh headings (keywords), cancer site, and canned search categories.

8

Monitor utilization by remote site to evaluate the frequency and demand for various types of educational content to permit refinements and revisions to improve offerings.
The MCN gathers extensive information in regards to use of the MCN website. This information includes website traffic, which asset was accessed, time spent, keywords searched for, the number of presentations watched, for credit or not, and the frequency with which each presentation is watched.

Task 5.   Design and create a web page to deliver physician referral information that includes submission electronic case record consisting of text and imaging data. (Months 1-36)

Develop and implement a database to archive text and imaging data for retrieval by consulting Cancer Center physicians and integration with Moffitt Cancer Center clinical information systems.
Moffitt has a DICOM server which, when combined with secure Internet protocols, may be used to transmit and receive DICOM-compliant images to and from partners on the Internet. These images are securely relayed to and from Moffitt's PACS viewing stations. This technology has been proven to work in experiments with the Haley Veterans' Administration Hospital and Cornell University. Radiology is currently working with Morton Plant Hospital to develop a permanent, Internet-based method of exchanging patient radiographic images. This system is currently used for all patients of Moffitt.

Develop a structured computerized clinical case description that provides a minimally relevant set of data that describes a clinical case for second opinion and consultation.
Efforts to date have focused on image transfers and the capability to be DICOM compliant. Appropriate mechanisms have been developed along with interfaces to hospital PACS and Radiology Departments.

Additional efforts focus on Clinical Genetic electronic pedigrees for use in remote case conferencing. This is expected to expand greatly in the coming year as the use of telemedicine for clinical genetics increases.

Acquire hardware and software to provide audio and video real time and time shifted streaming of case conferencing to remote locations for user viewing over secure communication links.
In July 2000 MCN procured rack mounted dual processor servers and audio/video equipment for the purpose of providing both real-time streaming of media as well as simultaneous capture of that media for archive.

In December 2000 MCN began exploring the use of low-cost, low bandwidth one-way and two-way case-conferencing equipment. This equipment would allow the patient to contact and conference with their respective physician without leaving their home. A preliminary trial of the equipment and its functionality, conducted in 2001, was successful.

In October 2001 MCN began streaming a monthly genetics case conference to our affiliate hospitals.

In 2002 MCN evaluated the use of a synchronous conferencing system called Lotus Sametime for the monthly genetics case conference. We believe this will improve the efficacy of the conference and by allowing remote users to interact with the geneticist.

The Lotus Sametime system along with the Microsoft Windows Media system has been extended not only into clinical genetics but also into various large scale international research programs.

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Establish the necessary gateways and bridges to provide connections at a range of bandwidths to support remote connectivity.
All processes are controlled remotely and designed for live to archive times of no more than five minutes. In other words, five minutes after a live broadcast event is completed, an "On-Demand" rebroadcast will be available to specific users. The former being broadcast via secure port and virtual link and the latter are encrypted for use with a specific key.

The Lotus Sametime System implemented in 2002 acts as gateway for limited traffic. A gatekeeper has been established to handle all other IP based traffic. These support bandwidths range from 32k to 3MB per second.

Design and implement web-based front ends to Moffitt Cancer Center clinical systems to permit secure access to patient information of patient's referred or submitted to case conferencing or second opinions.
Moffitt is in the process of a Cerner Clinical record system implementation. Upon the completion of this project, restricted access to case information could be made available via the web.

Task 6.   Provide access to case conferencing from remote locations using easily available audio/video to the desktop. (Months 1-48)

Complete telegenetics experiment to assess feasibility and acceptability of this format for the exchange of clinical information.
The telegenetics experiment has been completed. Findings are as follows:

Of 74 eligible subjects, 60 agreed to participate. There were no differences in previous technology exposure between the 14 who declined participation and the 60 who agreed. Of the 60 participants, 30 received their initial genetic counseling session via telemedicine, and 30 received the session face-to-face. There were no differences perceived in patient or provider satisfaction between the face-to-face and telemedicine pre-test sessions. Of the 60 participants, 23 proceeded with genetic testing, 12 of whom had received the initial session face-to-face and 11 via telemedicine. Based on the crossover design of the study, these 23 individuals received their post-testing genetic counseling session via the opposite method from their initial session. A two-sided test of significance showed no difference in overall satisfaction between telemedicine and face-to-face sessions.

A new telegenetics study is being designed to be submitted to the NCI National Community Clinical Oncology Program. This study is intended to explore the feasibility of delivering cancer susceptibility genetic counseling and testing services via telemedicine. We anticipate randomizing 200 participants.

Each individual who presents for cancer genetic counseling (having been referred through their health care provider or through self-referral) will be recruited for the study, until study accrual has been completed.

Each individual will be asked to participate in the study by agreeing to be randomized to receive genetic counseling via one of two methods 1) face-to-face (standard) or 2) telemedicine. As part of the study explanation, candidates will be provided with an introduction to telemedicine by physically seeing the equipment and being allowed to "try it out" by briefly visualizing and talking with another individual via

the connection. This "hands-on" introduction should ensure informed consent for the study. Patients who elect to participate in the study will sign a written informed consent form.

All individuals who have agreed to be randomized for the study will be asked to complete the State-Trait Anxiety Inventory evaluating their pre-test genetic counseling anxiety levels. This serves as a baseline and is administered after an individual is ascertained to be at increased risk for familiar cancer, but before the individual has undergone genetic counseling.

A BRCA1 & 2 knowledge questionnaire consisting of 11 true-false measures, which include items used in core instrument was developed for use by the National Center for Genome Research (CHGR) Cancer Studies Consortium. This instrument will be used to assess differences in knowledge transfer between the two delivery modalities.

One week after the conclusion of the post-test genetic counseling session, the Strait-Trait Anxiety Inventory and the knowledge instrument will be administered to the patient via telephone to assess differences in anxiety levels that may exist between the face-to-face and telemedicine consultations.

Implement additional sites to expand this program and resolve billing issues within the context of existing laws and regulations regarding telehealth and teleconsultation programs.
A preliminary structure has been put in place for support, however legal limitations existing within the state hinder rapid progress on developing a large scale clinical program. New legislation is expected during the next two years and we are working with other institutions to address billing, reimbursement and practice issues.

The genetics department has extended the reach of the genetics case conferencing program into ten statewide centers and continues to explore adding case conference members.

Establish the necessary gateways and bridges to provide connections at a range of bandwidths to support remote connectivity.
MCN is implementing two strategies, traditional videoconferencing and Sametime web based videoconferencing at the desktop. Sametime provides a low cost, secure mode of communication, primarily aimed at researchers and M.D.s involved in case conferencing. Traditional videoconferencing provides a widely adopted videoconferencing modality and therefore use of the equipment does not necessarily mean the adoption of new technology for remote centers.

Develop tunneling or other secure links to resolve firewall issues regarding LAN configurations at both the Moffitt Cancer Center and remote sites.
Moffitt is using Virtual Private Networks with key fob and biometric authentication technology. A Cisco Multimedia Conferencing Server is being placed in tandem with the firewall to provide a secure single point of access through the firewall for videoconferencing.

Acquire and install technology in conference centers where case conferencing generally occurs for selected clinics to permit retrieval and display of multiple images and clinical data submitted for this purpose by remote users.

For each site, a detailed plan of operations has been developed to establish the capability to schedule and transmit signals for MCN distribution. MCN has implemented streaming equipment for Clinical and Research presentations given at the center.

MCN technology has been applied to the conferencing centers in the new Moffitt Clinic and Research buildings. The buildings are equipped to allow for automated capture of educational assets from any conference room within the facility. The cost of this was absorbed as part of the overall construction cost to Moffitt. Money has been budgeted in the current year to bring a percentage of the non-MCN equipped conference rooms up to the same standards as the newly equipped room in the new facilities.

In March 2001, MCN successfully completed the installation of case conferencing equipment in two primary conference centers in the main building and the original research center building.

Plans for a fully interactive case conferencing center are in progress. The center would provide access to digital radiology, relevant patient information, and a host of other technologies.

Assess utilization of this technology to refine and revise formats and improve the quality and ease of remote access.
MCN has made it a priority to improve the quality of its products. Moving towards the use of Microsoft products and its MPEG-4 streaming format has reduced labor and increased quality across the board. MCN has implemented programs for remote control of streaming servers. A migration to XML took place in June of 2002, improving portability of the system. The use of scan converters in capture of educational content has greatly improved quality of the final asset. Finally, changes in its business practices have reduced labor requirements and increased quality and functionality as well as increased the customer base.

MCN continues to work to increase capability and functionality, improving video quality, and lowering bandwidth requirements for the user, while at the same accruing more content and reducing the production time by streamlining and automating the process.

Sametime videoconferencing was implemented to provide low cost case conferencing access to smaller, possibly rural medical centers.

The MCN outfitted conferencing facilities in the newly constructed buildings to produce an end-to-end digital image and greatly increases the overall quality of the assets produced.

KEY RESEARCH ACCOMPLISHMENTS:

●The Moffitt Cancer Network is available to users and can be found at
http://network.moffitt.usf.edu
●The MCN currently has 576 presentations in its library. Additionally, 16 conferences sponsored by USF and Moffitt are also currently available online.
●All approved Grand Rounds presentations have been taped by the Moffitt Multimedia Education Resources Center (MERC) for over two year preceding this report. The video was previously captured on digital DVCAM 94 minute tapes. Currently we are running in a tape-less environment.

•Since many of the presenters use only 35mm slide for their presentations, a process of creating final production audio/video Real media for streaming via TCP/IP has been developed. This process requires post-production labor and requires the best of the video's individual frames to be captured a second time to recreate higher quality computer images. MCN has made significant progress in this area and as of June 2000 has begun using presenter's PowerPoint files when ever possible to bypass the second image rendering process. This has reduced labor time from 3.5 days to about 5 hours, while increasing image quality noticeably. This labor savings is not realized when presenters are using 35mm film only. This methodology was modified to capture slides, overheads and computer screens digitally without a camera. The new methodology has reduced post-production time to virtually nothing. This allows us to concentrate on acquisition of new material.

•In addition to pre-presentation file acquisition, MCN has begun the development of a presenter packet. When finished, this packet will inform presenters to repeat important questions asked at the end of events like Grand Rounds and these will be added to the content to be available to medical professionals at the MCN website.

•National oncology conferences have been taped and included in the MCN website database. •Conferences have been subdivided into their respective presentations and are categorized searchable as well as searchable using the website database Access Jet engine. All conferences are pre-qualified for their ability to become online educational materials by the University of South Florida College of Medicine and, more recently, the University of South Florida College of Nursing.

•MCN began simultaneous live streaming and archiving in late 2001. This process greatly reduces postproduction time while increasing access to live events.

•MCN has completed the move to camera-less and tape-less acquisition of presentations using a host of digital equipment.

REPORTABLE OUTCOMES:

•Patents and licenses applied for and/or issued;

   A notice of disclosure has been filed with the USF office of patents in anticipation of the completion of a patent application.

•Presentations

   • The Moffitt Cancer Network Vision, Jeffrey Krischer, Ph.D. April 2001
   • The Moffitt Cancer Network, Lessons Learned and New Directions, Matthew Clark, B.S. October 2001
   • The Moffitt Cancer Network 2002, Matthew Clark, B.S. April 2002
   • Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., May 19, 2002 Medical Library Association Conference, Dallas TX
   • No-latency video architecture, efficiency and a new tomorrow for on-line education, Matthew Clark, B.S. June 2002
   • Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., June 19, 2002 Tech Topics, Moffitt
   • Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., October 19, 2002 Southern Chapter, Medical Library Association
   • Disseminating Library Instruction to the Desktop via the Web, Sue Felber, M.S., October 19, 2002 Southern Chapter, Medical Library Association
   • Telemedicine Today and Tomorrow, Matthew Clark, B.S. October 2002

•Abstracts
- J Permuth-Wey, JA Betts, AB Cantor, JP Krischer, R Sutphen: Cancer Genetic Counseling and Testing by Telemedicine - Results of a Feasibility Study (Abstract). American Journal of Human Genetics (2002) 71(4): 343.

CONCLUSIONS:
The purpose of this research is to create processes that allow medical professional to extend their abilities through the use of electronic media. MCN has evolved in pace with the change of that technology and because of its foresight and its dedication to purpose it has kept ahead of the technology. MCN has realized that streaming media processes are now capable of high definition presentations at low bandwidths and has developed the best possible processes for producing usable educational media delivery using network technology. MCN's research into these processes has revealed the need for specific products and their uses. Several new programs have been developed to address these processes. For example, to cut down on the need for many new employees, MCN has developed a broadcast program that will allow a single user to set start/stop times on a given event at a given location.

Conventional videoconferencing has limitations of cost and support while not meeting security and privacy requirements of HIPAA. Lotus Sametime may be a cost effective means of HIPAA complaint case and video conferencing. Providing second opinion and expert information to referring physicians is an extremely important piece of MCN's research. While continuing education is a given, in the final analysis, it may be in the medical professional interaction that MCN becomes most useful. If it were determined effective Sametime would provide a secure, cost effective case conferencing system that would allow smaller and rural centers as well as individual doctors a means to gain access to Moffitt expertise.

This experience has led to the demonstration of efficient and effective web-based video-conferencing methodologies. The environment regarding the presentation of lectures, case conferences and lecture programs has proven effective and well accepted by health care providers. Future plans have led to the integration of this technology in other research programs facilitating education, communication and data sharing. These applications have built upon the technology base developed by the MCN and have led to independent funding to support specific research projects.

REFERENCES: None

Appendix C

The Tampa Bay Ovarian Cancer Study

Rebecca Sutphen, M.D.

Tampa Bay Ovarian Cancer Study
Principal Investigator: Rebecca Sutphen, M.D.

1. Introduction

The BRCA1 and BRCA2 genes are believed to account for the majority of inherited ovarian cancer, yet few population-based studies have been performed specifically to investigate their role in this deadly disease – there are no population-based reports from the U.S. The largest population-based study to date was done in Ontario, Canada*, based on 649 unselected cases of ovarian cancer, of which 515 were invasive. Methodology for mutation detection included screening of exon 11 of BRCA1 and exons 10 and 11 of BRCA2 through protein truncation test. In addition, all samples were screened for 11 common mutations by rapid multiplex method (including 3 Jewish founder mutations and 6 French Canadian mutations). Utilizing this limited mutation detection strategy, among the 515 women with invasive ovarian cancer, 60 mutations (11.7%; 95% Confidence Interval: 9.2-14.8%) were identified, among which 39 mutations were identified in BRCA1 and 21 mutations were identified in BRCA2. The average age at diagnosis of BRCA1 carriers, BRCA2 carriers, and sporadic cases was 51.2, 57.5, and 56.7 years, respectively. Of the 60 mutation carriers, 38 patients (63%) had a positive family history of breast and/or ovarian cancer, and 22 patients (37%) did not. Pathologic analysis showed that 56 of the 60 (93%) mutation carriers had invasive serous cancers, and the remaining 4 women had endometrioid tumors.

The Tampa Bay Ovarian Cancer Study (TBOCS) established a coalition of investigators to perform a population-based case-case study of incident epithelial ovarian cancer in a heavily populated 2-county region of west central Florida. This coalition, including regional community physicians, was used to accrue incident cases of ovarian cancer diagnosed between December 13, 2000 and September, 30, 2003, through a rapid case ascertainment mechanism in Hillsborough and Pinellas counties, Florida, including the greater Tampa - St. Petersburg - Clearwater metropolitan area with a population in excess of 2 million. Through this study, 231 women diagnosed with ovarian cancer between the ages of 18-80 were enrolled. In-person interviews were conducted with all subjects, in order to collect comprehensive data on health behaviors, risk factors, personal and family history; provide genetic counseling; and obtain blood samples. Complete sequencing of the BRCA1 and BRCA2 coding regions was performed to allow assignment of cases (mutation-carriers) and controls (non-carriers) and determination of the prevalence of BRCA1 and BRCA2 germline mutations in this population.

The aims of this study of incident epithelial ovarian cancer are:

1) to investigate whether and which health behaviors and risk factors differ between germline mutation-associated cases and non-mutation controls;

2) to examine differences in the family cancer history profile of mutation-associated cases and non-mutation controls;

3) to examine differences in tumor characteristics between mutation-associated cases and non-mutation controls;

4) to investigate differences in response to treatment and survival between mutation-associated cases and non-mutation controls;

5) to achieve an 80% participation rate.

2. Body

The study was reviewed by the Surgeon General's Human Subjects Research Review Board (SGHSRRB) on September 27, 2000. Final approval to open the study for enrollment was obtained on December 13, 2000.

*Risch, HA, McLaughlin, JR, Cole, DEC et al. Prevalence and Penetrance of Germline BRCA1 and BRCA2 Mutations in a Population Series of 649 Women with Ovarian Cancer. Am.J.Hum.Genet. 68: 700-710, 2001.

Data Compilation of currently available data on 231 participants enrolled in the study is summarized in table 1 below:

Table 1

Information	Number Available	Number Pending
All TBOCS paticipants (n=231)		
BRCA1 and BRCA2 mutational analysis	210	21
Pathology Report	171	60
Tumor Blocks Collected	150	81
Ethnicity Data	189	42
Family History Information	173	58
Menopausal Status	138	93
Mutation Carriers (n=31)		
Pathology Report	29	2
ER/PR Status	22	9
Menopausal Status	13	18

Status of tasks included in the approved statement of work is as follows:

Task 1: Preparation for Medical Record Abstractions

Data elements of the medical record abstraction form were finalized. Design of the medical record abstraction form and the data entry mechanism for pathology data were completed. This design allows direct data entry of abstracted medical records into the database, followed by independent review of the medical record data by the pathologist at the time of pathology analysis. This mechanism made data entry highly efficient while ensuring data quality.

Task 2: Recruitment and Training of Study Personnel

We enrolled 231 ovarian cancer patients in this study through their treating physician. Data from the Florida Cancer Datasystem shows that in women diagnosed with invasive epithelial ovarian cancer between age 18-80, ~85% of regional cases are diagnosed by 7 gynecologic oncologists, hence recruitment strategies involved recruiting all gynecologic oncologists in the region as co-Investigators and training their staff regarding the study. This first strategy was accomplished, except that one of the 7 gynecologic oncologists refused participation, despite several strategies to facilitate participation, hence only a limited number of his patients could be enrolled in this study. Through several strategies used to centralize responsibility for patient contact to study staff, we were able to decrease responsibilities of local physicians' staff, which facilitated enrollment.

Task 3: Subject recruitment and Data Collection

Patient recruitment for this study involved the treating gynecologic oncologist introducing the TBOCS study to their patients with incident invasive epithelial ovarian cancer, and if patients were agreeable to study participation, the study team was informed. A genetic counselor scheduled an in-person meeting with the patient at the gynecologic oncologist's office, or at another convenient location for the patient. The study interview was a successful strategy to accomplish explanation of the study, provision of informed consent, enrollment, completion of study questionnaire, genetic counseling, and blood sampling. Medical records have been successfully obtained for all recruits on whom they have been requested, however there are outstanding medical records on 60 recently recruited women, and these records are in the process of being obtained. Tumor tissue has been successfully obtained from the appropriate surgical locations for pathology analyses on 150 patients thus far. Tumor blocks are in the process of being collected on the remaining, more recently recruited, TBOCS participants. Paraffin embedded tumor block specimens have been successfully obtained on every patient on whom they were requested, and all remaining samples on the recently recruited women will be collected within the next few months. Blood samples

have been successfully obtained for genetic testing and banking for future research on all women recruited into the study.

Data on the initial 113 patients enrolled in the study has been extracted and show a distribution of histological subtypes, stage, and median age of diagnosis similar to that seen in the general population (refer to table 2):

Table 2: Description of the data from the first 113 TBOCS patients.

	Expected number based on general population	Actual number seen in TBOCS study (n=113)
Histology:		
Serous	75%	72% (81/113)
Endometrioid	15-25%	16% (18/113)
Stage Distribution:		
Stage I/II	30%	26% (29/113)
Stage III/IV	70%	74% (84/113)
Median Age:	63	59

The two most populous counties within the Moffitt catchment area are Hillsborough and Pinellas, which account for ~75% of the population, and were the counties from which TBOCS participants were recruited. Data has been compiled on the initial 189 TBOCS patients, and shows that 55% are from Hillsborough and 45% are from Pinellas. Table 3 summarizes the catchment area demographics, the distribution of cancer cases for this area and the comparable distribution of cases seen through TBOCS. Demographic data are culled from the 1990 census; incident cancer case data for the catchment area is from the State Cancer Registry 1998 data. The distribution of TBOCS patients by race/ethnicity is similar to the distribution of cancer cases in the catchment area, as shown in the table below (Table 3).

Table 3. Ethnic Background of TBOCS patients compared to demographics Tampa Bay Region

Race /Ethnicity	Catchment Area Demographics	Catchment Area Cancer Cases	TBOCS patients (N=189)
White/Not Hispanic	84%	91%	89.5%
Hispanic	5.9%	2.8%	6.4%
Black/Not of Hispanic origin	9%	4.7%	1.6%
American Indian	0.2%	0.0%	1.6%
Asian/Pacific Islander	0.9%	0.3%	0.5%
Other		0.0%	0.5%
Unknowns		1.1%	

Task 4: Disclosure of results to the patients

The results of genetic testing and related information (depending on results) were provided to subjects who elected to receive results. Of the 231 women recruited, only 1 woman elected not to receive results. Results of genetic testing were utilized for assignment of case-control status and matching. Results are currently available on 210 of these women. Of the first 210 women enrolled in the study, 31 (14.8%) had mutations in BRCA1 or BRCA2: 20 in BRCA1 (9.5% of cases) and 11 in BRCA2 (5.2%).

The mutations in the BRCA1 gene were distributed throughout the 5 regions of the gene, as shown in Table 4. Of the BRCA2 mutations, 45% (5/11) were outside the Ovarian Cancer Cluster Region (OCCR), as shown in Table 5. The higher rate of mutation detection compared to the Ontario study was, in part, due to comprehensive analysis performed in this study.

Table 4: BRCA 1 mutations (n=20)

Specific BRCA1 Mutation	Region of BRCA1
2530delAG	3
C944X	3
2576delC	3
187delAG	1
3790ins4	4
5385insC	5
187delAG	1
4154delA	4
4440insG	4
E1134X	3
3875del4	4
187delAG	1
K679X	2
2800delAA	3
187delAG	1
187delAG	1
C61G	1
2800delAA	3
2576delC	3
1294del40	2

Table 5: BRCA2 mutations (n=11)

Specific BRCA2 Mutation	OCCR of BRCA2
Q1931X	yes
Q2009X	yes
2041insA	no
R2520X	no
6174delT	yes
4512insT	yes
1983del5	no
E49X	no
6174delT	yes
R2520X	no
4706del4	yes

The percentages of patients having a positive family history of breast and/or ovarian cancer in a first or second degree relative for BRCA1 carriers (n=20), BRCA2 carriers (n=11), and women with sporadic ovarian cancer (n=142) were 65%, 82% and 30%, respectively. The average ages of diagnosis for these 3 groups of women were 53, 58, and 59, respectively. Personal and family history information on cancer diagnosis has been compiled on 31 mutation carriers and 142 sporadic cancers and is shown in table 6 below:

Table 6: Personal and family history of cancer in participants

Family History	BRCA1 (n=20) 65%	BRCA2 (n= 11) 35%	BRCA+ (n= 31)	Sporadic (n=142)
Negative family history of breast or ovarian cancer in 1st or 2nd degree relative	n=7 (35%)	n=2 (18%)	n=9 (29%)	99 (70%)
Positive family history of breast or ovarian cancer in 1st or 2nd degree relative	n=13 (65%)	n=9 (82%)	n=22 (71%)	43 (30%)
Personal history of breast and ovarian cancer	n=6 (30%)	n=3 (27%)	n=9 (29%)	0 (0%)
Age at diagnosis of ovarian cancer				
≤40	0 (0%)	1 (9%)	n=1 (3%)	15 (10.6%)
41-50	8 (40%)	2 (18%)	n=10 (32%)	21 (14.8%)
51-60	10 (50%)	2 (18%)	n=12 (39%)	25 (17.6%)
>60	2 (10%)	6 (55%)	n=8 (26%)	39 (27.5%)
Range	42-77	34-73	34-77	33-80
Mean	53	58	54	59

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This data compares to the limited population-based data available from published reports, showing that family history may not predict germline BRCA1 and BRCA2 mutations in a substantial proportion of carriers. Additionally, the mean age of onset of ovarian cancer in BRCA1 carriers is reported to be about 5 years earlier than that seen BRCA2 carriers. In BRCA2 carriers, mean age at diagnosis is similar to that seen in the general population, consistent with previous reports.

Task 5: Abstraction of Medical Records

Of the 231 women in this study, medical record abstractions have been completed for 171 enrolled subjects (ie: 142 sporadic cancers and 29 mutation carriers) and 6 month, 12 month and 24 month follow-up data has been obtained for appropriate participants. Data abstracted from medical records includes review of histologic subtype, grade, and stage of cancer and is shown in the tables below (table 7 and table 8).

Table 7: Data from medical record review of carriers

Histology	BRCA1 (n=18) *	BRCA2 (n=11)	BRCA+ (n=29)*	Sporadic (n=142)
Serous	12 (67%)	5 (45.5%)	17 (58.6%)	62 (43.7%)
Endometrioid	1 (5.5%)	2 (18.2%)	3 (10.3%)	15 (10.6%)
Transitional	1 (5.5%)	0 (0%)	1 (3.4%)	6 (4.2%)
Mucinous	0 (0%)	0 (0%)	0 (0%)	5 (3.5%)
Mixed	4 (22%)	1 (9.1%)	5 (17.2%)	8 (5.6%)
Peritoneal	0 (0%)	1 (9.1%)	1 (3.4%)	0 (0%)
Brenner	0 (0%)	1 (9.1%)	1 (3.4%)	0 (0%)
Unknown	0 (0%)	1 (9.1%)	1 (3.4%)	0 (0%)

* data is not available on 2 of the 20 BRCA1+ women

As seen in table 7, most of the tumors had serous histology, and none were mucinous or borderline tumors, similar to previous literature reports. In addition, of the 22 mutation carriers on whom this data has been compiled, all BRCA1- and BRCA2- associated ovarian cancers were ER-/PR- and ER+/PR+ respectively, consistent with previous reports.

Table 8: Stage and Grade Information in BRCA1 and BRCA2 carriers

	BRCA1 (n=18) *	BRCA2 (n=11)	BRCA+ (n=29)*
Stage			
I	1 (6%)	4 (36.4%)	5 (17.2%)
II	2 (11%)	1 (9.1%)	3 (10.3%)
III	13 (72%)	5 (45.5%)	18 (62.1%)
IV	2 (11%)	1 (9.1%)	3 (10.3%)
Grade			
1	4 (22%)	3 (27.3%)	7 (24.1%)
2	2 (11%)	1 (9.1%)	3 (10.3%)
3	12 (67%)	7 (63.6%)	19 (65.5%)

* data is not available on 2 of the 20 BRCA1+ women

As seen in table 8, the distribution by stage of BRCA carriers was similar to that seen in the general population, where ~15% present in stage 1 and 70% present in stages 3 or 4. However, 17% (3/18) of BRCA1 carriers compared to 45.5% (5/11) of BRCA2 carriers presented with early stage disease (ie: stage 1 or 2), with a p-value of 0.08, based on the Fisher's Exact Test. We suspect that this borderline significant value is due to the small number (ie: 3) of observed early stage BRCA1 carriers.

Task 6: Tumor Tissue Analyses

Tumor tissue has been collected for the first 150 recruits thus far, and is in the process of being collected for the remainder. Pathology analyses are being completed and tissue will then be banked for future research.

Task 7: Follow-up for Survival

Follow-up data at 6 months, 12 months and 24 months has been obtained as appropriate. Follow-up contacts will continue for surviving enrolled subjects after 6 months, after 1 year, and annually as funding permits. Data entry and quality control measures are ongoing. Data on the initial 100 women recruited into the study indicates that 15 women have died thus far.

Task 8: Statistical Analyses and report writing

Interim analyses include acceptance for publication in *Cancer Epidemiology, Biomarkers, and Prevention*, of the data arising from the lysophospholipids screening study (Appendix A). Interim analyses of the first 100 women enrolled in TBOCS were presented at the 2002 American Society of Human Genetics annual meeting (Appendix B). Interim analyses of the first 164 women enrolled in TBOCS has been accepted for presentation at the 2003 Frontiers in Cancer Prevention Research annual meeting of the American Association for Cancer Research (Appendix C). Final analyses and reports are in the process of being prepared, pending final data collection.

3. Key Research Accomplishments

Aim 1: Data regarding health behaviors and risk factors were obtained from all participants via questionnaire instruments and study interview was successfully completed on all 231 women. Data regarding menopausal status has been compiled on 138 women. Of the 13 mutation carriers on whom data has been compiled, 0, 4, and 9 are pre-, peri-, and post-menopausal, respectively. Of the 125 sporadic cases on whom data has been compiled, 17, 26, and 82 are pre-, peri-, and post-menopausal, respectively.

Aim 2: Detailed cancer family history was obtained from all participants via questionnaire instruments and study interview.

Aim 3: A successful mechanism has been implemented to obtain medical records and tumor tissue in order to compare tumor characteristics between mutation-associated cases and non-mutation controls.

Aim 4: A successful follow-up mechanism has been implemented to obtain data regarding differences in response to treatment and survival between mutation-associated cases and non-mutation controls.

Aim 5: Although we were not able to achieve an 80% participation rate, we have been able to accrue a population-based sample with regard to ethnicity, stage, histologic subtype, median age and ethnicity (refer to Tables 2 and 3). Based on Florida Cancer Data System (FCDS) data, we estimate that a total of 430 patients were diagnosed with ovarian cancer during the study period, of whom 350 patients were ascertained (ie: 82%). There were 12 patients who died prior to enrollment, 18 patients or doctors declined, and a further 10 patients who could not be enrolled prior to the closing of the study. Hence of the 350 cases ascertained, 231 were enrolled in the study (66%).

4. Reportable Outcomes

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was awarded by the American Cancer Society for a companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer (7/1/00 – 6/30/04). Preliminary data is promising and shows that certain lysophospholipids appear to be elevated in the plasma of women with ovarian cancer compared with healthy controls (article accepted in Cancer Epidemiology, Biomarkers, and Prevention and included in Appendix A). We have applied to ACS for a two-year extension of the project. Also, based on this preliminary data, we have applied to NIH for R01 funding to investigate the use of lysophospholipid measurement and proteomic profiles for detection of ovarian cancer in a case-control study.

Based on data showing that gene mutations associated with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are the third leading cause of hereditary ovarian cancer (after BRCA1 and BRCA2), and the suggestion that ovarian cancer is a "sentinel cancer" in individuals with these gene mutations, an investigation of HNPCC as a companion study of TBOCS has been funded.

The preliminary results of this research were presented at the 2002 American Society of Human Genetics annual meeting (Appendix B), and 2003 Frontiers in Cancer Prevention Research (American Association for Cancer Research) annual meeting (Appendix C).

5. Conclusions

Epithelial ovarian cancer results in the death of more American women than all other gynecologic cancers combined. The Tampa Bay Ovarian Cancer Study was the first U.S. population-based study to evaluate the role of the BRCA1 and BRCA2 genes in the etiology, pathology and response to treatment of this deadly disease. Findings from this study include: 1) The incidence of hereditary ovarian cancer due to BRCA1 and BRCA2 mutations is higher than previously reported; 2) BRCA2 mutations account for a higher percentage of hereditary ovarian cancer cases than previously reported; 3) Previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR); 4) approximately 30% of hereditary ovarian cancer patients have no obvious family history to suggest hereditary cancer susceptibility, hence family history may not be sufficient to accurately predict mutations. A companion study to assess the potential of biologically active lysophospholipids as biomarkers in ovarian cancer was funded and is in progress. The Tampa Bay Ovarian Cancer Study and its companion study represent an important opportunity to evaluate the role of inherited susceptibility to ovarian cancer and evaluate lysophospholipids for their potential as biomarkers of this deadly disease.

APPENDIX A

Lysophospholipids Are Potential Biomarkers of Ovarian Cancer

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<u>Lysophospholipids Are Biomarkers of Ovarian Cancer</u>

<u>Abstract</u>

Objective

To determine whether lysophosphatidic acid (LPA) and other lysophospholipids (LPL) are useful markers for diagnosis and/or prognosis of ovarian cancer in a controlled setting.

Method

Plasma samples were collected from ovarian cancer patients and healthy control women in Hillsborough and Pinellas counties, Florida, and processed at the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida (Moffitt). 117 case patients with epithelial ovarian cancer and 27 healthy control subjects participated in the study. Blinded LPL analysis, including 23 individual LPL species, was performed at the Cleveland Clinic Foundation, using an electrospray ionization mass spectrometry-based method. LPL levels were transmitted to Moffitt where clinical data were reviewed and statistical analyses performed.

Results

There were statistically significant differences between preoperative case samples (N=45) and control samples (N=27) in the mean levels of total LPA, total lysophosphatidylinositol (LPI), sphingosine-1-phosphate (S1P) and individual LPA species, as well as the combination of a number of LPL species. The combination of 16:0-LPA and 20:4-LPA yielded the best discrimination between preoperative case samples and control samples, with 93.1% correct classification, 91.1% sensitivity and 96.3% specificity. In 22 cases with both preoperative and postoperative samples, the postoperative levels of a number of LPL, including S1P, total LPA and LPC levels and some individual species of LPA and LPC, were significantly different from preoperative levels.

Conclusion

LPA, LPI, LPC and S1P appear useful as diagnostic and prognostic biomarkers of ovarian cancer.

Introduction

The mortality rate for women with ovarian cancer is very high, with an estimated 14,300 deaths from ovarian cancer in 2003 in the United States.[1] More than two-thirds of patients have late stage metastatic disease at initial diagnosis with a 5-year survival rate of approximately 20 - 30%.[1-4] Conversely, at early stages, the long-term survival rate approaches 90%.[5] There is currently no proven effective method for early detection of ovarian cancer through biomarkers, imaging, or other means. The most common biomarker for ovarian cancer, CA 125, lacks specificity and is elevated in only about 50% of stage I ovarian cancer cases.[3, 4, 6] Proteomic patterns derived from surface-enhanced laser desorption/ionization mass spectroscopy analysis have recently shown promise for early ovarian cancer detection[7] but further studies regarding their reproducibility and reliability for early detection and screening are needed.

Lysophosphatidic acid (LPA) has been proposed as a sensitive biomarker.[8] However, studies investigating the utility of LPA as a biomarker for early detection of ovarian cancer have yielded conflicting results. Preliminary findings from a study which included 48 healthy controls and 48 women with ovarian cancer showed that plasma LPA levels (measured by gas chromatography) were elevated in patients with ovarian cancer ($P<0.001$).[8] Importantly, elevated levels were detected in early-stage ovarian cancers compared with controls.[8] The study also compared available CA125 values with LPA levels and results suggested that plasma LPA may be a more sensitive marker for ovarian cancer, particularly for stage I disease.[8] A recent Korean study of only 3 pairs of samples also showed differences between ovarian cancer cases and controls.[9] However, in another study where LPA levels were measured in plasma samples from 32 patients with ovarian cancer and 32 healthy controls using a liquid chromatography / mass spectroscopy assay, results showed no significant elevation in plasma LPA levels in

ovarian cancer patients compared to controls, raising questions about the utility of plasma LPA levels for early detection of ovarian cancer.[10]

LPA is present in the ascitic fluid of patients with ovarian cancer[11, 12] and may function as an autocrine factor, contributing to ovarian cancer proliferation, cell survival, angiogenesis and metastasis.[13-22] Lysophosphatidylinositol (LPI), a related LPL to LPA, has also been found at increased levels in ascites fluid and plasma of ovarian cancer patients compared with controls[23] and has been shown to display signaling properties in cellular systems.[24, 25] Thus, LPI may also have utility as a biomarker of ovarian cancer, and data suggests that measuring LPI in addition to LPA may increase the sensitivity and/or specificity of the test.[23] Both LPA and LPI represent various subspecies with different fatty acid chains. In addition, the fatty acid chain may link to the glycerol backbone through different chemical linkages resulting in various subclasses (i.e., acyl- (LPA), alkyl- (A-LPA), and alkenyl- (An-LPA). Findings of a study to evaluate the discriminating ability of LPA and LPI *subspecies* for ovarian cancer identification compared with *total* LPA and LPI suggested that subspecies with unsaturated fatty acid chains may be associated with late-stage or recurrent ovarian cancer.[26] Other LPL that have been proposed to have a biologic role in ovarian cancer and be potentially useful as biomarkers of the disease include lysophosphatidylcholine (LPC), which has also been shown to be elevated in the plasma of ovarian cancer patients[27], and the lysosphingolipid sphingosine-1-phosphate (S1P) which is known to have both extracellular and intracellular signaling properties.[28-31]

To further explore the potential of LPA, LPI, LPC and S1P as biomarkers for ovarian cancer detection, we measured plasma LPL levels (including subspecies of LPA, LPI and LPC) in women with ovarian cancer and healthy controls, using an electrospray ionization mass spectrometry (ESI/MS) method recently developed by Dr. Xu's group at the Cleveland Clinic

Foundation.[23] This assay allows simultaneous detection and quantitation of different species of LPL with at least 10 times more sensitivity than the previous gas chromatography method.[23]

Materials and Methods
Patients

All patient-derived biologic specimens were collected under protocols approved by the Institutional Review Board of the University of South Florida and all participants provided written informed consent.

Whole blood samples were obtained preoperatively in EDTA tubes by routine venipuncture of women undergoing surgery for suspected ovarian cancer in Hillsborough and Pinellas counties, Florida between December 13, 2000 and October 30, 2002. All women ages 18 – 80 undergoing surgery for suspected ovarian cancer in the two counties during the defined period were regarded as eligible for entry into the study. No patients who were asked refused to participate. Of the preoperative samples obtained, 45 were from women who were later confirmed to have ovarian cancer or primary peritoneal cancer (ovarian cancer patients) (median age 60 years, range 33-79). Samples were obtained postoperatively from ovarian cancer patients from the same eligibility pool (N=94, median age 59, range 26-80), including 22 patients who had contributed a preoperative sample and 72 who had not. Whole blood samples from control subjects were collected concurrently from healthy women from the same counties who reported no history of cancer, gynecologic disease, oophorectomy or family history of breast/ovarian cancer (N= 27, median age 45, range 22-79). Whole blood specimens were obtained from a total of 117 ovarian cancer patients, including 18 patients with stage I disease, 11 with stage II disease, 74 with stage III disease and 14 with stage IV disease. Among the 45 patients for whom a preoperative sample was available, there were 7 patients with Stage I disease, 3 with Stage II disease, 31 with stage III disease and 4 with stage IV disease. Cancer diagnosis was confirmed for all cases by review of pathology records by a single ovarian cancer expert. Clinical stage was

determined according to International Federation of Gynecologists and Obstetricians criteria[32], and the histologic subtype was evaluated according to the World Health Organization classification.[33]

Sample Collection

LPA is produced and released by activated platelets during coagulation and therefore is a normal constituent of serum, but it is present only at very low levels in whole blood or fresh platelet-poor plasma from healthy individuals.[8] To prevent platelet activation and phospholipase activity, blood samples were collected in EDTA-containing tubes. Since LPLs are metabolites and levels may change during incubation, it is important that sample processing be as consistent as possible across all samples for comparison. We collected samples from multiple locations in the two study counties and processed (centrifugation and aliquotting) all samples at the Moffitt Cancer Center. After blood drawing, samples were immediately chilled for transport to Moffitt by being placed in a styrofoam container accompanied by a frozen pack for overnight delivery. This system allowed centrifugation within 16-28 hours after blood drawing. Centrifugation was at 3000g for 20 minutes after which the plasma was immediately aliquotted per each 0.5 cc into coated microEppendorf tubes and immediately frozen at $-70°C$. Samples were batch-shipped on dry ice by overnight delivery to the Cleveland Clinic for analysis. Shipped samples were identified by a unique sample number only, without identifiers or any indication of the subject's status as ovarian cancer patient or control. The samples were maintained at $-70°C$ until preparation for mass spectrometry analysis. No personnel at the Cleveland Clinic had knowledge of the subjects' disease status at any time. Laboratory data was transmitted according to each unique sample number to the Moffitt Cancer Center where all statistical analyses were performed.

LPL Analysis

Lipids were extracted as described previously with minor modifications.[23, 34] To 0.5 mL plasma, 2 mL of MeOH/chloroform (2:1) and 0.1 mL of 6 N HCl were added. Samples were vortexed for 1 min and incubated on ice for 10 min. 1 mL of chloroform and 1 mL of H2O were added to separate the phases. Samples were vortexed for 0.5 min prior to centrifugation (2,000 g for 10 min). The lower phase was transferred to a new glass tube. To the upper phase left in the original tube, 1 mL of chloroform was added to extract more lipids and the tube was centrifuged (2,000 g for 10 min). The lower phase was transferred into the same tube (with the lower phase extract) and the solvent was evaporated under nitrogen at 30°C. The dried lipids were suspended in 50 µL of solvent (MeOH:chloroform, 2:1), vortexed, and applied to a thin-layer chromatography (TLC) plate. Two standards (18:1-LPA and 18:1-LPC) were applied to help in identifying the "LPA band" and the "LPC band" on each TLC plate. The TLC plates were developed in the solvent system (chloroform:MeOH:AmOH, 65:35:5.5) until the solvent front was 1.5 inch from the top of the plate. The lipids from the "LPA band" and the "LPC band" were eluted with 2 mL of MeOH:chloroform (2:1) twice. The lipid solutions were dried under nitrogen at 30°C and lipids resuspended in 100 µL of MeOH for mass spectrometry.

Mass spectrometry analyses were performed using a Quattro Ultima triple quadrupole ESI-MS (Micromass, Inc., Beverley, MA) with the Masslynx data acquisition system. A Waters 2690 (Waters) autosampler was used to introduce the samples into the ESI source. The mobile phase used for all experiments was MeOH:H2O (9:1; v:v) and the flow rate was 100 µL/min. The injection volume was set to 20 µL/sample for all experiments. The positive or negative ion-mode with multiple reaction monitoring (MRM) was used to quantitatively analyze the positively or negatively charged phospholipids. The collision energies were 70 eV in the negative mode and 25 eV in the positive mode. Nitrogen was used as both drying and nebulising gas at flow

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rates of 500 L/h and 50 L/h, respectively. The ESI probe capillary was held at 3 kV for the positive mode and *3 kV for the negative mode and the cone voltage was set at 35V in positive mode and *50 V in negative mode. The source and desolvation temperatures were 100°C and 200°C, respectively.

LPA and other negatively charged LPL were analyzed in the negative mode with the monitoring ions at m/z 378 (parent ion) - 79 (product ion) for S1P, 381-79 for 14:0-LPA, 393-79 for 16:0- Alkenyl- LPA, 395-79 for 16:0 -Alkyl- LPA, 409-79 for 16:0-LPA, 421-79 for 18:0-Alkenyl-LPA, 423-79 for 18:0-Alkyl-LPA, 433-79 for 18:2-LPA, 435-79 for 18:1-LPA, 437-79 for 18:0-LPA, 571-79 for 16:0-LPI, 599-79 for 18:0-LPI, and 619-79 for 20:4-LPI, respectively. All lipids with the phosphorylcholine group (positively charged) were analyzed in the positive mode. Monitoring ions were at m/z 465 (parent ion) - 184 (product ion) for SPC, 496-184 for 16:0-LPC, 510-184 for 17:0-LPC, 520-184 for 18:2-LPC, 524-184 for 18:0-LPC, 544-184 for 20:4-LPC and 568-184 for 22:6-LPC, respectively. The dwell time in the MRM mode was 0.11 ms and the scan delay was 0.02s.

Statistical analysis

Categorical variables were analyzed using Chi-square tests or Fisher's exact tests, depending on sample size. Continuous variables, including univariate comparisons for quantitative variables between normal and cancer cases, were compared using the Student's t-tests, or the Wilcoxon Rank Sum test, depending on the distribution of the variable of interest. Adjustment for potential confounding variables, such as the stage at diagnosis, was carried out by using general linear modeling or analysis of variance methods, as appropriate. Stepwise logistic regression analysis was used to determine the statistical significance of LPA, LPI, LPC (and their subspecies) and S1P. All statistical significance testing was 2-sided, and P values less than .05 were considered to be statistically significant. P values in the range of .01 to .05 should

be interpreted with caution because of multiple testing issues. Statistical analyses were performed utilizing SAS Software, SAS Institute Inc, Cary, NC.

Results

The ages, stages, grades, histologic subtypes and treatment status of the 117 ovarian cancer patients who participated in the study are shown in Table 1. A total of 166 samples were analyzed, including 27 from healthy controls, 45 obtained preoperatively from women with ovarian cancer and 94 obtained postoperatively from women with ovarian cancer, with 22 patients having both preoperative and postoperative samples.

There were statistically significant differences between preoperative case samples (N=45) and control samples (N=27) in the mean levels of several individual LPA species, the combination of 16:0-LPA/20:4-LPA, total LPA, total LPI and S1P (Table 2). The best discrimination between samples obtained preoperatively from ovarian cancer patients and those from healthy controls was achieved by the combined levels of 16:0-LPA and 20:4-LPA , with 93.1% correct classification, 91.1% sensitivity and 96.3% specificity (Figure 1). Receiver operating characteristic curves (ROC)[35] were examined and a cutoff 16:0-/24:0-LPA level of 0.62 µM was identified as optimizing the sensitivity and specificity of the assay (Figure 1). All patients with preoperative samples had 16:0-/24:0-LPA levels above the 0.62 µM cutoff, with the exception of one stage I patient, one stage II patient and 2 stage III patients. Using an ROC-derived cutoff value of 1.5 µM, total LPA levels achieved 91.7% correct classification, 91.1% sensitivity and 92.6% specificity (Figure 2). All 4 of the cases which had 16:0-/20:4-LPA levels below the 0.62 µM cutoff also had low total LPA levels, as might be expected since total LPA includes 16:0-LPA and 20:4-LPA. Similarly, the control with an elevated 16:0-/20:4-LPA level of 0.91 µM also had the highest total LPA level.

The mean values for the combination of 16:0-LPA/20:4-LPA in the plasma samples obtained preoperatively from patients with stage I, stage II, stage III and stage IV ovarian cancer were 1.23 µM (S.D. 0.52), 0.92 µM (S.D. 0.43), 1.23 µM (S.D. 0.70) and 0.93 µM (S.D. 0.15), respectively, compared with 0.35 µM (S.D. 0.17) for the controls (Table 2). The mean values of total LPA in the plasma samples obtained preoperatively from patients with stage I (7 patients), stage II (3 patients), stage III (31 patients) and stage IV (4 patients) ovarian cancer were 2.57 µM (S.D. 0.94), 2.15 µM (S.D. 0.71), 2.93 µM (S.D. 1.77) and 1.97 µM (S.D. 0.27) µM, respectively, compared with 0.90 µM (S.D. 0.43) for 27 healthy controls (Table 2). The mean values of total LPI in the plasma samples obtained preoperatively from patients with stage I, stage II, stage III and stage IV ovarian cancer were 2.98 µM (S.D. 1.57), 4.58 µM (S.D. 2.71), 4.25 µM (S.D. 2.81) and 2.96 µM (S.D. 0.33), respectively, compared with 1.51 µM (S.D. 0.79) for the controls (Table 2).

In 22 cases with both preoperative and postoperative samples, the postoperative levels of total LPA, total LPC, 22:6-LPA, 18:0-LPA, the combination of 20:4-LPA/22:6-LPA, 20:4-LPC and 18:2-LPC were significantly lower than preoperative levels (P= .03, .05, .02, .04, .03 .02, .003 and .03, respectively) (Table 3). Of these LPL, 18:0 LPC, 18:2 LPC and total LPC levels also showed statistically significant differences between preoperative case samples (N=45) and all postoperative case samples (N=94) ($P \leq .05$).

Discussion

Ovarian cancer is a disease associated with a high mortality mainly because it currently escapes detection at early stages. Identification of an effective biomarker for early detection would improve survival. This study reports documents statistically significant differences in LPL levels between preoperative samples of ovarian cancer patients and those of healthy

controls. The study also confirms that statistically significant elevations in LPL levels are present in patients with early stage disease. Thus, the findings support the utility of LPL, especially LPA, as biomarkers for early detection of ovarian cancer. The study is the first to report significant postoperative changes in specific LPL levels, suggesting that some LPL may also have utility as biomarkers of recurrence. The study also contributes data toward determination of the best combinations of markers and cutoff values for clinical use.

Although our conclusions are still preliminary because our study sample is small and not ideal for demonstrating the value of LPL for screening, our findings regarding the utility of LPL as biomarkers of ovarian cancer are critically important, since the two previous studies showed conflicting results.[8, 10] In order to ensure the validity of our data, only investigators at Moffitt had access to clinical data and the investigators performing LPL measurements at Cleveland Clinic were blinded to the case versus control status of the samples. All statistical analyses were performed at Moffitt.

The reason for the discrepancy between the findings of the two prior studies with interpretable results regarding the utility of LPA as a biomarker for detection of ovarian cancer is unclear. There were many methodologic differences between the two studies, including differences in sample collection, processing and lipid analyses.[8, 10] Our experience suggests that it is critical to maintain consistency of procedures for all samples to be compared, including the time and temperature prior to and during centrifugation, sample storage vials (see below), extraction solvents and methods, establishment of standard curves and mass spectroscopy methods. The following example demonstrates the importance of these aspects. Prior to analyzing the samples included in this report, we analyzed a batch of samples (N=33) that showed lower overall LPL levels than anticipated among both cases and controls, with less

separation than anticipated between levels of cases and those of controls. These findings prompted a review of procedures. Our review identified that the type of microEppendorf tubes used for storage after centrifugation was critically important. If the tubes were not siliconized or prelubricated, as much as 90% of negatively charged LPL were absorbed into the tube walls. Further analysis was performed, including paired storage of identical samples using coated and uncoated tubes, with the resulting differences in LPL levels analyzed. The analysis confirmed that the difference in tubes accounted for the differences in levels observed; therefore data from these samples was not included in the analyses (data not shown). The following suggestions are offered for future investigations of LPL: we recommend using the SafeSeal Microcentrifuge Tubes, Catalog #505-201 (PGC Scientifics, Frederick, MD) for plasma storage, and use of glass ware only (not plastic ware), except for the storage tubes mentioned above.

Further studies are underway to evaluate specificity of LPL measurements obtained not only from healthy controls, but also from women with benign gynecologic disease, other gynecologic cancers and non-gynecologic cancers. Additional studies are planned to evaluate LPL measurements in combination with other markers, including proteomic markers[7] and algorithms of changes in CA 125 values over time.[36] Longitudinal data will allow us to evaluate whether and when specific LPL return to baseline after successful treatment, and their utility in predicting recurrence. Studies are also needed to specifically address the utility of LPL measurements in women at hereditary risk for ovarian cancer, a group in whom early detection is desperately needed, but in whom baseline LPL levels may differ from healthy women at average risk (unpublished preliminary data). Thus, larger studies with the capability of yielding more precise estimates of the sensitivity and specificity of LPL, both alone and in combination with other markers for both screening and detection of recurrence are necessary.

In summary, our findings support the potential of LPL levels as biomarkers of ovarian cancer - specifically LPA levels as diagnostic markers and LPC as prognostic markers. However, these findings require validation in larger studies.

Acknowledgements

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Figure 1

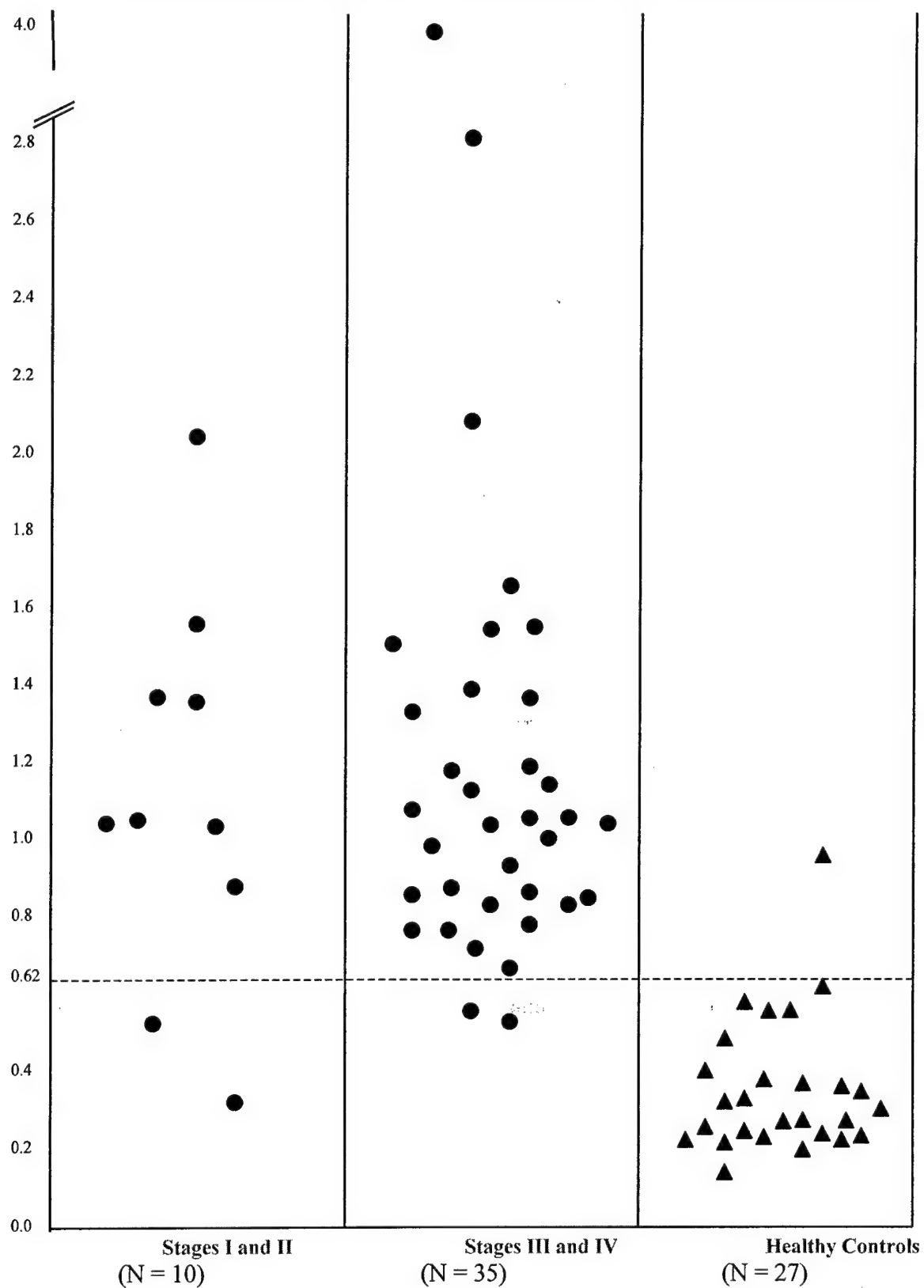16:0-LPA/20:4-LPA Levels (μM) in Preoperative Case Samples and Controls

| Stages I and II | Stages III and IV | Healthy Controls |
| (N = 10) | (N = 35) | (N = 27) |

Figure 2

Total LPA Levels (μM) in Preoperative Case Samples and Controls
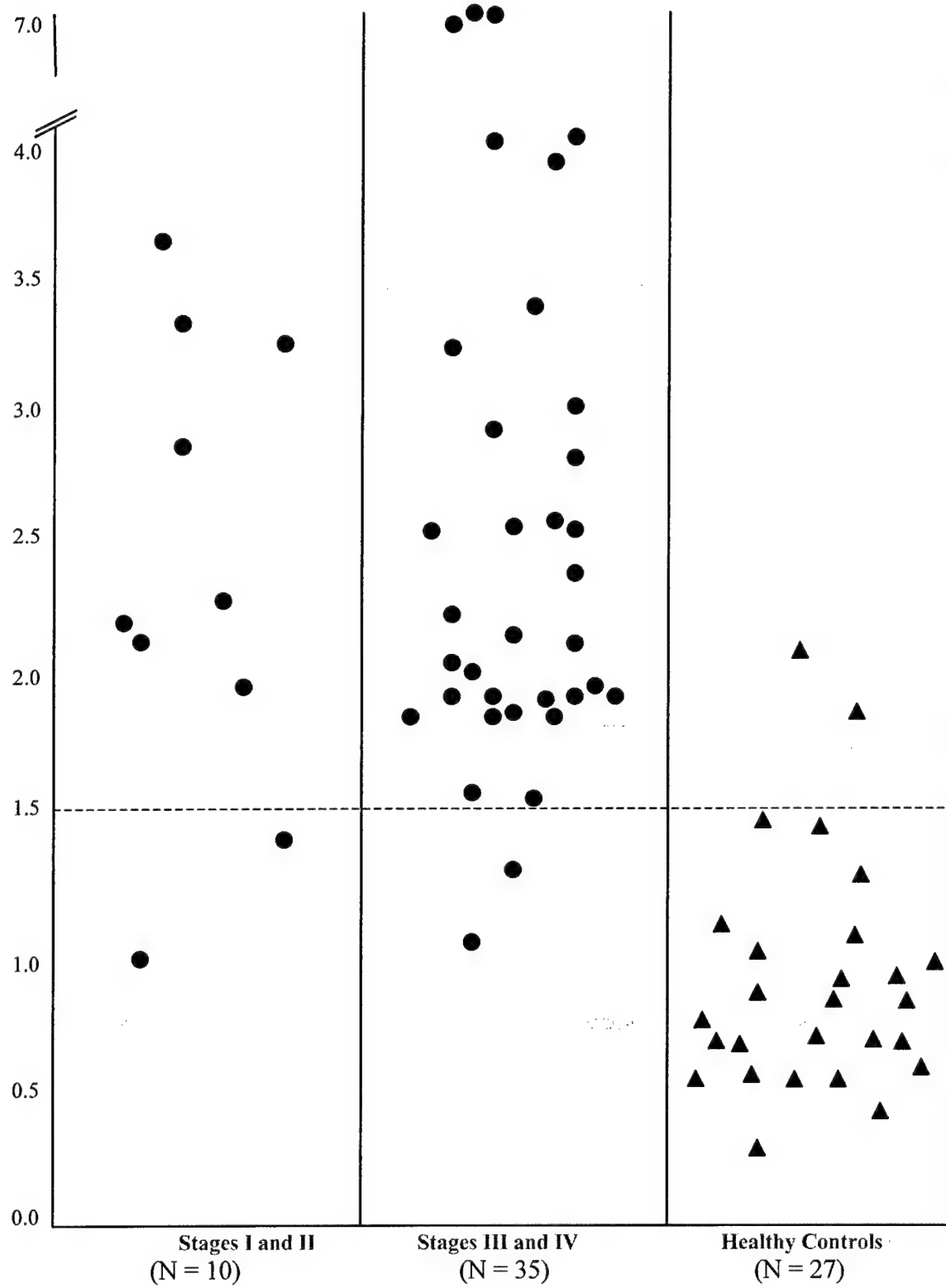
<div align="center">

Table 1
Clinical Data for Patients with Ovarian Cancer
(N = 117)

</div>

Characteristics	Stages I and II (N = 29)	Stages III and IV (N = 88)
Age, median (range), yr	60 (32 – 77)	59 (26 – 80)
Stages		
I	18 (15.4%)	-
II	11 (9.4%)	-
III	-	74 (63.2%)
IV	-	14 (12.0%)
Grades		
1	10 (8.5%)	11 (9.4%)
2	08 (6.8%)	21 (17.9%)
3	11 (9.4%)	55 (47.0%)
Ungraded	00	01 (0.9%)
Histologic types		
Serous	12 (10.3%)	61 (52.1%)
Endometrioid	11 (9.4%)	07 (6.0%)
Mixed	00	08 (6.8%)
Mucinous	03 (2.6%)	02 (1.7%)
Primary Peritoneal	00	04 (3.4%)
Clear cell	02 (1.7%)	02 (1.7%)
Transitional cell	01 (0.9%)	02 (1.7%)
Brenner	00	02 (1.7%)
Treatment status		
Pre-operative	10 (8.5%)	35 (29.9%)
Post-operative	19 (16.2%)	53 (45.3%)

Table 2

Means (Standard Deviations) for LPL in Controls and Preoperative Case Samples by Stage
(in μM)

Substance	Controls (N = 27)	Stage I (N = 7)	Stage II (N = 3)	Stage III (N = 31)	Stage IV (N = 4)
16:0-LPA ++++	00.14 (00.13)	00.52 (00.39)	00.62 (00.35)	00.73 (00.73)	00.37 (00.14)
18:0-LPA ++++	00.13 (00.10)	00.47 (00.42)	00.29 (00.19)	00.53 (00.51)	00.23 (00.03)
18:1-LPA ++++	00.17 (00.14)	00.37 (00.27)	00.46 (00.29)	00.47 (00.36)	00.32 (00.06)
18:2-LPA ++++	00.16 (00.14)	00.29 (00.26)	00.31 (00.08)	00.46 (00.39)	00.34 (00.09)
20:4-LPA ++++	00.22 (00.16)	00.71 (00.47)	00.31 (00.13)	00.50 (00.31)	00.55 (00.17)
22:6-LPA +++	00.09 (00.07)	00.20 (00.12)	00.16 (00.09)	00.24 (00.24)	00.16 (00.03)
16:0-A-LPA +	00.11 (00.08)	00.15 (00.07)	00.08 (00.05)	00.18 (00.08)	00.19 (00.04)
18:0-A-LPA ++	00.04 (00.06)	00.07 (00.08)	00.10 (00.06)	00.08 (00.06)	00.07 (00.03)
16:0-An-LPA ++++	00.07 (00.05)	00.18 (00.11)	00.11 (00.01)	00.15 (00.10)	00.17 (00.05)
18:0-An-LPA ++++	00.03 (00.04)	00.07 (00.03)	00.11 (00.06)	00.09 (00.07)	00.04 (00.03)
Total A-LPA ++++	00.25 (00.12)	00.48 (00.13)	00.40 (00.10)	00.50 (00.19)	00.47 (00.04)
Total LPA ++++	00.90 (00.43)	02.57 (00.94)	02.15 (00.71)	02.93 (01.77)	01.97 (00.27)
16:0-LPA/20:4-LPA ++++	00.35 (00.17)	01.23 (00.52)	00.92 (00.43)	01.23 (00.70)	00.93 (00.15)
16:0-LPI +++	00.49 (00.47)	00.75 (00.59)	01.88 (01.34)	01.00 (00.64)	00.90 (00.23)
18:0-LPI +++	00.50 (00.43)	00.87 (00.71)	01.77 (02.49)	01.89 (02.05)	00.70 (00.25)
20:4-LPI ++++	00.51 (00.43)	01.35 (00.78)	00.93 (00.95)	01.36 (00.84)	01.36 (00.24)
Total LPI ++++	01.51 (00.79)	02.98 (01.57)	04.58 (02.71)	04.25 (02.81)	02.96 (00.33)
16:0-LPC	52.37 (25.63)	70.65 (30.07)	55.98 (26.57)	52.98 (30.62)	48.10 (21.15)

Table 2 (con't)

Means (Standard Deviations) for LPL in Controls and Preoperative Case Samples by Stage
(in μM)

Substance	Controls (N = 27)	Stage I (N = 7)	Stage II (N = 3)	Stage III (N = 31)	Stage IV (N = 4)
18:0-LPC	15.63 (08.28)	21.00 (09.90)	17.23 (10.98)	14.90 0(9.56)	14.81 (06.57)
18:1-LPC	16.89 (07.27)	21.71 (10.42)	18.97 (13.40)	17.06 (11.40)	17.61 (10.02)
18:2-LPC +	20.21 (07.63)	17.50 (07.72)	16.63 (12.86)	15.12 (08.99)	16.34 (10.36)
20:0-LPC	00.21 (00.07)	00.25 (00.12)	00.19 (00.08)	00.33 (00.41)	00.20 (00.14)
20:4-LPC	10.44 (03.10)	11.60 (04.95)	09.38 (01.56)	10.11 (04.72)	10.36 (03.41)
22:6-LPC +	05.89 (02.24)	10.41 (06.00)	06.98 (04.63)	08.56 (05.96)	09.65 (05.96)
Total LPC	121.65 (47.22)	153.12 (60.02)	125.37 (68.84)	119.07 (64.40)	117.05 (57.06)
S-1-P +++	00.36 (00.27)	00.77 (00.42)	00.50 (00.43)	00.66 (00.48)	00.65 (00.26)

P values show significance levels for differences observed between healthy controls (N = 27) and all ovarian cancer cases for whom preoperative samples were available (N = 45).

+ $P< .05$
++ $P< .01$
+++ $P< .001$
++++ $P< .0001$

Table 3

Means (Standard Deviations) for Paired Preoperative and Postoperative Samples
(N = 22)

Substance	Preoperative Mean	Postoperative Mean
16:0-LPA	00.85 (00.84)	00.50 (00.28)
18:0-LPA +	00.64 (00.61)	00.33 (00.24)
18:1-LPA	00.55 (00.41)	00.36 (00.29)
18:2-LPA	00.39 (00.43)	00.38 (00.27)
20:4-LPA	00.55 (00.39)	00.47 (00.41)
22:6-LPA +	00.28 (00.28)	00.12 (00.09)
16:0-A-LPA	00.17 (00.09)	00.16 (00.15)
18:0-A-LPA	00.10 (00.06)	00.09 (00.10)
16:0-An-LPA	00.14 (00.07)	00.14 (00.11)
18:0-An-LPA	00.09 (00.07)	00.06 (00.07)
Total A-LPA	00.50 (00.18)	00.44 (00.27)
Total LPA +	03.27 (01.98)	02.16 (01.04)
16:0-LPA/20:4-LPA +	01.41 (00.78)	00.97 (00.51)
16:0-LPI	01.21 (00.91)	01.24 (01.40)
18:0-LPI	02.06 (02.32)	01.28 (01.37)
20:4-LPI	01.38 (00.99)	01.34 (01.06)
Total LPI	04.65 (03.21)	03.86 (02.05)
16:0-LPC	52.61 (30.34)	67.32 (36.06)
18:0-LPC	13.72 (08.62)	18.96 (10.18)

Table 3 (con't)

Means (Standard Deviations) for Paired Preoperative and Postoperative Samples
(N = 22)

Substance	Preoperative Mean	Postoperative Mean
18:1-LPC	15.08 (09.13)	20.95 (10.90)
18:2-LPC ++	13.95 (08.49)	21.67 (07.76)
20:0-LPC +	00.30 (00.43)	00.38 (00.64)
20:4-LPC	09.51 (04.68)	13.19 (05.32)
22:6-LPC	07.64 (05.69)	09.13 (04.77)
Total LPC +	112.81 (59.37)	151.60 (67.52)
S-1-P +	00.78 (00.54)	00.48 (00.29)

Statistically significant differences between preoperative mean values and postoperative
mean values are indicated as:

+ $P < .05$
++ $P < .01$

APPENDIX B

Abstract submitted to the annual meeting of the American Society of Human Genetics
(October 2002):

Tampa Bay Ovarian Cancer Study – A Population-based Study of BRCA1/2 in Ovarian Cancer.

Tuya Pal, Jeffery P. Krischer, Tricia Holtje, Judith A. Betts, Jenny Permuth Wey, James Fiorica, Edward Grendys, James LaPolla, Hector Arango, Katie Wakeley, Mitchell Hoffman, George Wilbanks, Santo Nicosia, Rebecca Sutphen and the Tampa Bay Ovarian Cancer Coalition

BRCA1 and BRCA2 are believed to account for the majority of hereditary ovarian cancers. Current estimates of mutation likelihood among ovarian cancer patients range from 9.2% (Myriad data) to 11.7% (Ontario population data, the only published population-based data).

To determine the prevalence, spectrum of mutations and genotype/phenotype correlations among ovarian cancer cases, we are conducting a population-based study of unselected incident cases of epithelial ovarian cancer in the geographic regions of Hillsborough and Pinellas counties, Florida (which includes Tampa, St. Petersburg, and Clearwater). Beginning in 2001, we have enrolled 100 women diagnosed with incident ovarian cancer, ascertained through their treating gynecologic oncologists. Medical records and tumor tissue have been reviewed and genetic counseling and DNA testing performed through full sequencing of the BRCA1 and BRCA2 coding regions and adjacent intronic base pairs.

Of the first 100 women enrolled in the study, 15 (15.0%) had mutations in BRCA1 or BRCA2: 7 in BRCA1 and 8 in BRCA2. No mutations were found among the 6 cases with mucinous tumors. No mutations were found among the 5 cases with borderline tumors; thus, the mutation frequency among invasive tumors was 15.8% (15/95).

These data suggest that 1) the frequency of BRCA1 and BRCA2 mutations among invasive ovarian cancer cases may be higher than previously reported, 2) previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR). Preliminary data regarding risk factors, penetrance, associated cancers and tumor characteristics is being analyzed and will also be presented.

APPENDIX C

Tampa Bay Ovarian Cancer Study: A Population-based Study of BRCA1/2 in Ovarian Cancer

Tuya Pal,[1] Jeffery P. Krischer,[1] Judith A. Betts,[1] Jenny P. Wey,[1] James Fiorica,[1] Edward Grendys,[1] Martin Martino,[1] James LaPolla,[2] Hector Arango,[3] Katie Wakely,[1] Mitchel Hoffman,[3] George Wilbanks,[3] Santo Nicosia,[3] Rebecca Sutphen.[1] Moffitt Cancer Center,[1] Tampa, FL, , Private Practice,[2] St.Petersburg, FL, University of South Florida,[3] Tampa, Florida.

BRCA1 and BRCA2 are believed to account for the majority of hereditary ovarian cancers. Current estimates of mutation likelihood among ovarian cancer patients based on the largest population-based data is 11.7% (Ontario population data). To determine the prevalence, spectrum of mutations and genotype/phenotype correlations among ovarian cancer cases, we are conducting a population-based study of unselected incident cases of epithelial ovarian cancer in the geographic regions of Hillsborough and Pinellas counties, Florida (which includes Tampa, St. Petersburg, and Clearwater). Beginning in 2001, we have enrolled 174 women diagnosed with incident ovarian cancer, ascertained through their treating gynecologic oncologists. Medical records and tumor tissue have been reviewed. Genetic counseling was provided and DNA testing was performed through full sequencing and evaluation for the 5 common large genomic rearrangements. Results are currently available on 164 of these women. Of the first 164 women enrolled in the study, 22 (13.4%) had mutations in BRCA1 or BRCA2: 12 in BRCA1 (7.3% of cases) and 10 in BRCA2 (6.1%). All BRCA1- and BRCA2- associated ovarian cancers were ER-/PR- and ER+/PR+ respectively. Of the BRCA2 mutations, 40% were outside the OCCR region. Most of the tumors had serous histology, and none were mucinous or borderline tumors. The percentages having a positive family history of breast and/or ovarian cancer in a first of second degree relative for BRCA1 carriers (n=12), BRCA2 carriers (n=10), and women with sporadic ovarian cancer (n=142) were 60%, 40% and 30% respectively. The average ages of diagnosis for these 3 groups of women were 54, 59, and 59 respectively.These data suggest that 1) the frequency of BRCA1 and BRCA2 mutations among invasive ovarian cancer cases may be higher than previously reported; 2) previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR); 3) it may be reasonable to offer any woman with an invasive non-mucinous ovarian tumor genetic counseling (up to 15-16% risk in this group); and 4) family history may not be sufficient to accurately predict mutations.

Appendix D

Personnel Receiving Pay from the Research Effort

Personnel Receiving Pay from the Research Effort

John Ruckdeschel
Jeffrey P. Krischer
Rebecca Sutphen
Nagi Kumar
Melvyn Tockman
Richard Jove
Carlos Muro-Cacho M.D.
Ping Dou
Emmanuel Lazaridis
Gail Shaw
Jong Park
Robert A. Clark
Dmitry G. Goldgof
Sudeep Sarkar
Wayne Cruse
Wei Qian
Claudia Berman
Alan Cantor
Judy Betts
Jenny Wey

Melissa Cochran, M.S.
Matthew Clark
Jamie Malloy
Keston Etienne
Margaret Grosos-King, R.N.
Marilyn Rogers
Mi,e Gruidl, Ph.D.
Shannon Terkowski, R.N.
Sherry Pace
Terry Diamond
Sai Ponduru
Glen Graham
Voula Harokopos
Dylan Lee
Kerry Nimmons
Pam Smith
William Smith
Jeff Szucs
Lan Chen